Staphylococcus aureus bacteremia — host factors influencing the human infection risk

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BY
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DISSERTATION SUBMITTED 2018

AALBORG UNIVERSITY
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Dissertation submitted

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TABLE OF CONTENTS

ABBREVIATIONS

SAB = *Staphylococcus aureus* bacteremia

SES = Socioeconomic status

IE = Infective endocarditis

PY= Person-years

SIR= Standardized incidence ratios

IRR= Incidence rate ratios

CI= Confidence interval

N/A= Not applicable

PREFACE

I first met my main supervisor Professor Christian Torp-Pedersen during my research year as a medical student (2012). You could speculate that Christian was not as impressed by my research skills as I was of him as a supervisor, because a few weeks after I became a PhD-student at Gentofte Hospital, Christian moved to Aalborg, thus almost as far away from Gentofte you can get without leaving Denmark. However, he accepted to be my main supervisor, which I am grateful for. Together with Professor Paal Skytt Andersen, Robert Leo Skov and Michelle Dalgas Skøtt Schmiegelow a strong group of supervisors was established. With the statistical expertise from Professor Per Kragh Andersen and Professor Thomas Alexander Gerds the group was close to perfect. I will like to address a special thanks to this group of people to whom I am deeply indebted. Without their tremendous knowledge, inspiration, tireless discussions, guidance, availability irrespective of time of day and support through these years, my PhD thesis would not have been realized. Michelle, I owe you extraordinary thanks! Besides being an excellent supervisor with a unique passion for this project you have become a very close friend. Thank you!

I owe a great thank you to all co-authors who have contributed to the work in this thesis and especially a great thanks to Andreas Petersen for answering endless questions regarding the Danish National *Staphylococcus aureus* bacteremia Database and for the massive help with the preparations of blood smears to the *spa* analysis in Paper III.

A grate full thanks to all my colleagues at Gentofte University Hospital. A special thanks goes to my indispensable office buddy and now close friend Anne-Christine Ruwald. It has been a privilege to work with such an inspiring, funny, helpful and intelligent colleague/friend. Also, thank you to Anna-Grethe Jensen for always having a full overview regarding paperwork, deadlines and for being extremely helpful.

To my family and friends – thank you for all your love and support, your interest in my work and your indulgence in hectic time periods. Mom – thank you for your irreplaceable love and support and for always reminding me that there is more to life than research. Dad – thank you for balancing the role as a loving father and a colleague with perfection.

Lastly, the ultimate thanks I owe to Morten – my best friend, my love, my husband and the most amazing father to our two children (Emilie and Victor). Thank you for your never-ending support in all aspects of life, for believing in me and loving me no matter what and in stressful times reminding me of what is essentially in life. You mean the world to me.

Louise Bruun Østergaard, April 2018

OVERVIEW OF PAPERS

Paper I

Oestergaard LB, Schmiegelow MD, Bruun NE, Skov RL, Petersen A, Andersen PS, Torp-Pedersen C. The associations between socioeconomic status and risk of *Staphylococcus aureus* bacteremia and subsequent endocarditis – a Danish nationwide cohort study. BMC Infect Dis. 2017 Aug 25;17(1):589. doi: 10.1186/s12879-017-2691-3. Pub-med PMID: 28841914

Paper II

Oestergaard LB, Schmiegelow MD, Gerds TA, Nygaard U, Bruun NE, Skov RL, Lauridsen TK, Dahl A, Petersen A, Andersen PS, Torp-Pedersen C. Staphylococcus aureus Bacteremia in Children Aged 5-18 Years-Risk Factors in the New Millennium. J Pediatr. 2018 Dec; 203:108-115.e3. doi: 10.1016/j.jpeds.2018.07.093. Epub 2018 Sep 21. PMID:30244992

Paper III

Oestergaard LB, Christiansen MN, Schmiegelow MD, Skov RL, Andersen PS, Petersen A, Aasbjerg K, Gerds TA, Andersen PK, Torp-Pedersen C. Familial Clustering of *Staphylococcus aureus* Bacteremia in First-Degree Relatives: A Danish Nationwide Cohort Study. Ann Intern Med. 2016 Sep 20;165(6):390-8. doi: 10.7326/M15-2762. Epub 2016 Jul 5. Pub-med PMID: 27379577

DANSK RESUMÉ (DANISH SUMMARY)

Blodforgiftning med gul Stafylokok (SAB) er en livstruende infektion, som rammer både børn og voksne over hele verden. En frygtet komplikation til blodforgiftningen er hjerteklapsbetændelse, hvoraf 30–40% af patienterne dør inden for et år. Flere risikofaktorer for SAB er identificeret blandt voksne, men der er fortsat uafklarede spørgsmål. Identifikation af faktorer som øger risikoen for SAB har stor betydning for muligheden for forebyggelse og tidlig opsporing af denne alvorlige sygdom. Hos voksne er det usikkert om socioøkonomisk status øger forekomsten af sygdommen, hvis alle har lige adgang til sundhedsvæsnet og det er uklart om social status påvirker risikoen for efterfølgende at få betændelse på hjerteklapperne hos patienter med SAB. Hos børn er viden om risikofaktorer for SAB mangelfuld, og eksisterende studier er præget af begrænsninger i forhold til design og få patienter. Det er ligeledes aldrig undersøgt om blodforgiftningen ophobes i familier. En familiær ophobning vil tyde på, en genetisk disposition til en øget modtageligheden over for bakterien gul Stafylokok.

Ved at sammenkoble landsdækkende registre via hver persons CPR-nummer indhentede vi oplysninger om bl.a. uddannelse, sygdomme, medicin, operationer, familierelationer og mikrobiologisk verificeret SAB for hvert individ.

Hovedresultaterne var: Artikel I) Hos voksne var lav socioøkonomisk status associeret med en øget forekomst af SAB, men sammenhængen blev svagere med stigende alder. Der var ingen association mellem socioøkonomisk status, og udvikling af hjerteklapsbetændelse hos patienter med SAB. Artikel II) Hos børn (5–18 år) var forekomsten af SAB højest blandt børn som modtog dialyse eller plasmaferese, var transplanteret, havde kræft, børneeksem, medfødt hjertesygdom eller var nyopererede, men mere end hver tredje barn med sygdommen var tidligere rask. For tidlig fødsel og forældrenes socioøkonomiske status påvirkede ikke forekomsten af blodforgiftningen. Artikel III) Raten af SAB var dobbelt så høj i familier, hvor en førstegradsslægtning havde haft sygdommen sammenlignet med baggrundsraten. Den højeste rate fandt vi, når ens søskende havde været syg og havde erhvervet infektionen uden for hospitalet. Smitte og miljøfaktorer var formentlig ikke forklaringen, da >80% af familierne var inficeret med genetisk forskellige gule Stafylokokker, og vi ikke observerede en øget forekomst af SAB fandt blandt ægtefolk.

Identifikation af risikofaktorer for SAB hos både voksne og børn (Artikel I og II) kan være med til at øge sundhedsfagliges opmærksomhed over for sygdommen og derved både bedre diagnosticering, sikre igangsættelse af tidlig behandling samt forbedre forebyggelse af SAB. Ligeledes kan viden om familiær ophobning (Artikel III) bane vejen for fremtidige genetiske studier for at øge forståelsen for sygdommen, samt potentielt identificere særlige risikogrupper, hvor sundhedsfaglige bør være særligt opmærksomme på sygdommen og eventuelt forebyggende tiltag.

ENGLISH SUMMARY

Staphylococcus aureus bacteremia (SAB) is of great concern worldwide due to the frequently fatal outcome, or endocarditis as a dreaded complication with a one-year mortality rate of 30–40%. Multiple factors increasing the risk of SAB have been identified, but whether socioeconomic status is associated with the adult risk of SAB in a society with a universal healthcare system or whether social status is associated with subsequent endocarditis in SAB patients is not fully elucidated. Further, knowledge on risk factors in the pediatric population is sparse and the current literature is limited by small numbers of patients and study design. Additionally, to the best of our knowledge a possible familial clustering of SAB has never been investigated. To improve future diagnosis, treatment and prognosis for patients with SAB, identification of exposures associated with increased rate of the disease, are essential to ensure early diagnosis. Moreover, a familial clustering of SAB would point towards a possible genetic component in the human susceptibility to *S. aureus*.

We cross-linked national registries at an individual level by use of each individual's personal identification number to obtain information on demographics, education, comorbidities, medication, surgical procedures, family relations, and microbiologically verified SAB.

The main results of this thesis were: Paper I) In adults, a low socioeconomic status was associated with an increased risk of SAB, and this association declined with advancing age. The level of socioeconomic status did not influence the risk of subsequent infective endocarditis in patients with bacteremia. Paper II) In children aged 5–18 years, the highest rates of SAB were observed in children receiving dialysis or plasmapheresis, transplanted children, children with cancer, congenital heart disease, atopic dermatitis and in children with recent surgery. However, more than every third child with bacteremia were presumably healthy prior to the hospital admission for SAB. Neither parental socioeconomic status nor prematurity were associated with the risk of SAB in these children. Paper III) Having a first-degree relative previously hospitalized with SAB was associated with a more than two-fold increased rate of the disease compared with the background population. The highest risk of acquiring the bacteremia was observed if a first-degree relative was a sibling with non-hospital acquired SAB. Contagion could probably not explain our findings, since the causative *S. aureus* strain differed genetically in more than 80% of the infected families, and no increased risk was observed in spouses.

Knowledge on risk factors for SAB in both adults and children (Paper I and Paper II) may increase the awareness of the disease, thus improving early diagnosis and correct treatment. Lastly, knowledge on familial clustering of the disease can pave the way for future clinical genetic studies leading to prevention of familiar clustering.

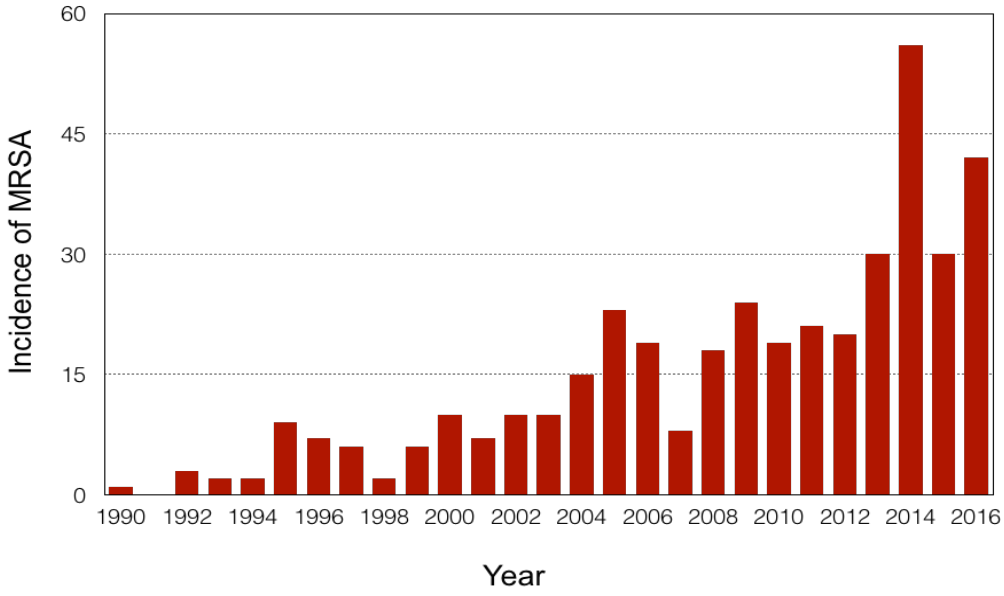
BACKGROUND

Staphylococcus aureus bacteremia (SAB) strikes people of all ages and may result in severe morbidity and mortality even in young and healthy individuals. The risk of SAB is influenced by multiple factors, and the interplay between host and microorganism is complex and far from elucidated. To prevent SAB and improve patient prognosis it is essential to understand to what extend non-genetic host factors are associated with an increased infection risk in both adults and children. Since genetic variation has also been implicated in increased susceptibility to *Staphylococcus aureus (S. aureus)* in animals and in a few human studies, it is also of great interest to investigate if the disease clusters in families as an indication of a possible genetic component in the human susceptibility to this pathogen.

Staphylococcus aureus (*S. aureus*)

S. aureus is a gram-positive bacterium which was first identified in 1880 in a knee joint and have ever since been a challenge to the healthcare system worldwide (1). The bacterium is a colonizing opportunistic pathogen residing on the human body and is a pathogen to man and other mammals. Approximately 20–25% of the Danish population are colonized *S. aureus* persistently in their nasal flora, and up till 50% are transient carriers (2,3). Besides bacteremia, *S. aureus* may cause a variety of diseases in humans ranging from superficial infections of the skin (e.g. impetigo and furuncles) to potential life-threatening diseases such as toxic shock syndrome, meningitis, endocarditis and osteomyelitis (4). To enter the host, *S. aureus* utilizes surface and virulence factors to damage the host tissue, block the innate immune response pathways and counteract the phagocytic engulfment (4). The pathogen is also known for its ability to colonize foreign bodies (e.g. pacemaker leads and joint prosthesis) with biofilm, thus infecting implanted body material while impeding the response from the immune system of the host (5). Further, in recent decades *S. aureus* has evolved to become multi-resistant to a large number of antimicrobial agents (6). In Denmark approximately 71–78% of all *S. aureus* strains are resistant to penicillin (7), whereas methicillin resistant *S. aureus* (MRSA) comprises only approximately 2% of the strains cultured in SAB patients, making the frequency of MRSA among SAB isolates very low compared with other countries such as the US where up to 60% of *S. aureus* infections in US intensive care units are caused by MRSA (8). Further, in Denmark an increased rate of MRSA (both carriers and infected people) has been observed in the community since 2000, Figure 1.

Figure 1: The incidence of new MRSA cases (carriers and infected individuals) in Denmark from 1990–2016

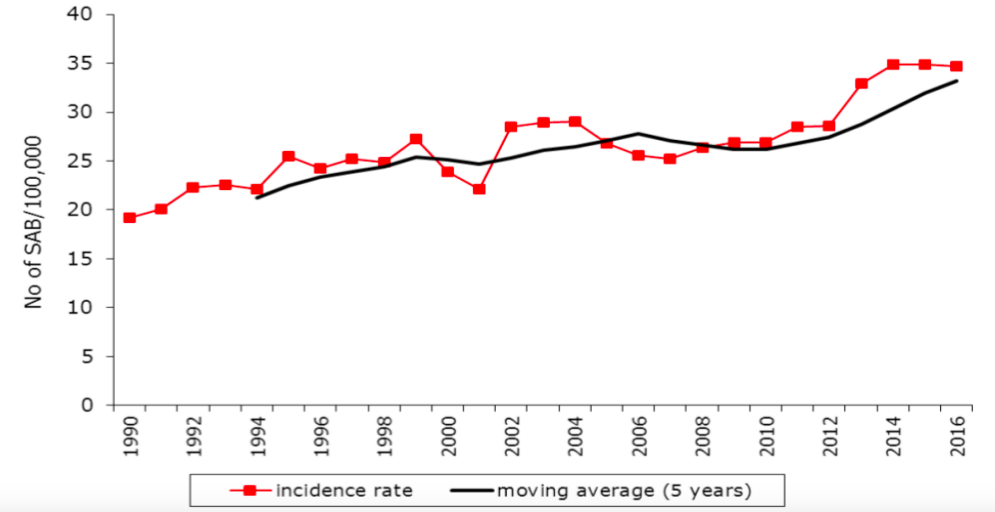


The figure is from the annual report on SAB with permission from the National Surveillance Department. Each patient only included one time unless found positive with a new strain type The increase in MRSA in 2014 might be explained by an increased screening for MRSA in pigs.

Staphylococcus aureus bacteremia (SAB)

Although intermediately fluctuating, in Denmark the incidence rate of SAB has increased continually over the past decades. In 2016 the annual incidence of SAB exceeded 34,7/100,000 inhabitants, Figure 2.

Figure 2: Incidence rate of SAB per 100,000 inhabitants from 1990–2016



The figure is from the annual report on SAB with permission from the National Surveillance Department

The bloodstream infection can be categorized according to the presumed acquisition (hospital acquired [HA], health-care related [HCR] and community acquired [CA]) and can occur as non-complicated or be complicated by e.g. endocarditis, orthopedic infections or meningitis (4,5,9–11), which prolong hospitalization and increase morbidity and mortality considerably (12–14). Nevertheless, the mortality rate in SAB patients has decreased gradually over the last couple of decades in Denmark (15,16).

Considering a Danish population with an increasing life expectancy, and a healthcare system, which continuously perform more invasive procedures, also in older patients, it is likely that the rate of SAB will continue to increase in the future. This only emphasizes the need for identification of risk factors to minimize doctor's delay and ensure an early diagnosis, and thus a better prognosis.

Host factors increasing the risk of SAB

The incidence rates of SAB have two age peaks, i.e. in the youngest (<1 year of age) and in older people (>75 years) (17–20). In children the occurrence of SAB is particularly high within the first year of life with a markedly higher rate among neonates (21,22), with premature birth and congenital heart disease as important contributing risk factors (22–25). During teenage years the rate of SAB declines markedly, remains low during adulthood and increase again with advancing age (2,3).

Although the reason remains unclear, studies consistently report male sex as an important risk factor for SAB. Among Danish patients with SAB, approximately two-thirds are males; a ratio that has been relatively constant over the last twenty years (16) and corresponds to what is observed elsewhere in the literature in both the adult and pediatric population (22,26).

Low level of socioeconomic status (SES) has been observed to increase the associated risk of SAB in adults in minor studies from Australia, Denmark and New Zealand (19,27,28). The associations are mainly explained by a higher prevalence of complicating life-style related comorbidities such as diabetes and cardiovascular diseases, as well as a larger occurrence of intravenous drug users. In countries without a universal health care system, disparity in the access to and quality of medical assistance increases the inequalities in acquisition and outcome of the

bacteremia. Despite the universal health care system in Denmark, also the Danish case-control study observed a low SES to be associated with an increased risk of community-acquired bacteremia in working-age citizens (27). Further, analyses stratified by pathogen revealed an inverse relationship between SES and SAB, but it is not fully elucidated if this relationship persists on a nationwide level including all SAB patients (27). It also remains to be shown if the level of SES in SAB patients is associated with subsequent infective endocarditis. In relation hereto, it has not been explored if parental SES is associated with the risk of SAB in children. A Danish study including children aged 0–5 years observed that children of parents on early retirement or of parents on sick leave were more likely to be hospitalized with an infectious disease compared with children of employed parents (29). Additionally, the researchers observed an inverse relationship between parental educational level and risk of offspring hospitalization. The association between SES and admission to hospital was strongest for ear infections, infection in the lower respiratory tract and gastrointestinal infections. Another Danish study examined the impact of socioeconomic factors on the risk of hospital admission due to an infectious disease within the first two years of life in children born in the Northern part of Jutland (30). Shorter or no maternal education, single mum status and low household income were associated with increased risk of hospitalization, but the association with low household income did not persist in adjusted models.

Several comorbidities have been observed to be associated with an increased risk of SAB, but only very few population-based studies include children and the majority hereof focuses on infants. Some shared common risk factors have been identified. Cancer is highly represented among both children and adults with SAB. Treatment with chemotherapy, surgery and long hospitalizations have a great impact on the infection risk (31–35). Further, a high occurrence of SAB is observed in patients with renal diseases especially when renal replacement therapy is required with the highest risk observed in patients undergoing hemodialysis (36–38). This has been confirmed in the pediatric population (39,40) and the high infection risk is partly due to repetetive intravenous access, central venous catheters and a suppressed immune system.

Other medical conditions prone to increase the disease risk are rheumatoid arthritis, which constitutes a significant risk factor for SAB in adults (41,42), and juvenile idiopathic arthritis in children (43,44). The inflammatory aspect of the diseases erodes the joints and in conjunction with immunosuppressive medication the body defense is impaired, rendering these patients more vulnerable to the pathogen. Studies on the relationship between SAB and inflammatory bowel disease are few in both the adult and pediatric population, and results are conflicting. But overall data suggest an increased rate of SAB. Immune suppressive and immune modulating medicine appear to play an important role (45–47).

Further, adults with diabetes have a substantially increased risk of the infection compared with non-diabetic individuals with poor glycemic control, duration of diabetes and complications to diabetes as major risk factors (48–52). These associations have, however, not been fully explored in children and studies show diverging results (53,54), as is the case for severe liver dysfunction, which in adults is associated with increased risk of the infection and a poor prognosis (51,55,56). Other risk factors for SAB are HIV (57–60) and injection drug-use (61–63), which are both rare in Danish children but is highly associated with SAB in the adult population.

Any invasive procedure and implantation of a foreign body is a risk factor of infections including SAB. The infection risk and type has been observed to differ according to type of implanted device (64,65). Moreover, patients receiving organ transplantation has an increased risk of SAB due to the invasive procedure, life-long treatment with immunosuppressive medication and the foreign material including scar tissue, (66,67).

Finally, nasal carriage has been shown to increase the risk of SAB (68,69). A number of studies have observed a higher risk of the disease among adult carriers compared with non-carriers and also that isolates obtained from blood cultures are clonally identical with isolates cultured from the nares in a large fraction of SAB patients (69–71). In newborns a study found maternal carrier status and antibiotics given during birth to be the most significant predisposing factors to neonatal *S. aureus* carriage (72). A study from Taiwan reported that 10,2% of otherwise healthy children carried MRSA with the highest carriage rate observed among infants (73).

Genes influencing the susceptibility to *S. aureus*

Although the section above describes multiple risk factors for SAB in both adults and children, it is not rare that a previously presumed healthy young adult or a child acquire SAB (10,25,74–76), raising the question if and to what extend host genetics contributes to the susceptibility to *S. aureus.*

Animal studies have shown that the susceptibility to *S. aureus* infection differs between different inbred mouse strains suggesting that genetic differences could also influence the susceptibility to *S. aureus* infection in humans. More specifically, it has been verified that certain types of inbreeded mice are more easily infected by *S. aureus* compared to other mice (77,78). Specific genetic changes on chromosome 7, 8, 11 and 18 have been shown to increase the susceptibility to *S. aureus* and further lead to an insufficient clearance of the infection by the immune system (79,80). Additionally, mice with a genetic deficiency resulting in impaired production of complement C5 had an increased susceptibility and increased mortality compared with healthy mice without C5 deficiency, when exposed to *S. aureus* (81,82).

In humans, the prevalence of SAB varies among genetically diverse ethnic populations (18,83–86). Studies have observed a doubling in incidence of SAB in the African-American population (66.5 per 100,000 person-years) compared with the Caucasian population (27.7 per 100,000 person-years) in the United States and an up to 20-fold increased risk in the Australian indigenous population (Aboriginals) compared with nonindigenous Australians (19,87,88). Likewise, in New Zealand the occurrence of SAB is more frequent among Pacific Island people and Maori compared with citizens of European ethnicity (85,89,90). Although SES influences the risk of SAB as outlined above, this cannot fully explain the inequality of *S. aureus* rates in these ethnic subpopulation (28) and, together with the aforementioned findings from animal studies, this suggests that host genetics may influence the susceptibility to *S. aureus* infection in these individuals, but this has yet to be demonstrated. It is well known that there is a significant heterogeneity in the health status of patients admitted with SAB, ranging from several comorbidities to none. Variation in host genetics could be a pathogenic factor in SAB patients. An increased sensitivity to *S. aureus* infections has been demonstrated in individuals suffering from rare genetic disorders, e.g. chronic granulomatous disease (91), IRAK-4 deficiency (92), Chediak-Higashis syndrome (93), hyper-IgE syndrome (94) and MyDD8 deficiency (95). A genome-wide association study explored whether common variants were associated with increased susceptibility to *S. aureus* but has failed to show convincing results (96).

If the susceptibility to *S. aureus* is partly driven by host genetics, it is reasonable to assume that SAB clusters in families, but this has not been explored previously.

Overall, a better understanding of human host genetic factors in relation to susceptibility to *S. aureus* infection is highly warranted.

OBJECTIVES AND HYPOTHESES

The overall purpose of this thesis was to identify human host factors associated with risk of SAB.

Paper I

The aim of this study was first to investigate whether the level of socioeconomic status (SES) was associated with the risk of a first episode of SAB in a nationwide setting, and secondarily if there was an association between SES and subsequent risk of infective endocarditis (IE) in patients with SAB. We *hypothesized* that low SES would be associated with both an increased risk of the disease as well as increased risk of subsequent IE in patients with SAB.

Paper II

The *aim* of this study was to utilize nationwide register data to investigate if predefined risk factors were associated with SAB in children aged 5–18 years i.e. in children with a mature immune system, and to identify presumably healthy children among children who developed SAB. We *hypothesized* that the absolute incidence rate of SAB in this pediatric population would be significantly lower than in studies following children from birth. We theorized that the majority of medical conditions and invasive procedures prone to increase the risk of SAB in the adult population would also be associated with increased risk of bacteremia in children, and that prematurity, parental SES and congenital disease would play an additional role. Lastly, we hypothesized that although the majority of children hospitalized with SAB would have a high comorbidity burden prior to the infection a significant proportion of the children would have a clean medical record.

Paper III

The *aim* of this study was to investigate whether a family history of SAB was associated with increased risk of the disease in first-degree relatives, and how potential associations differed according to family status (sibling, parent) and mode of acquisition. We *hypothesized* that first-degree relatives to individuals previously hospitalized with SAB would have an increased rate of the infection compared with the background population. We further hypothesized that the strongest association would be observed among first-degree relatives to an index-case with non-HA SAB. Lastly, we hypothesized that the association would be particularly strong among siblings.

METHODS

Data sources

The Danish society is unique due to its universal healthcare system, specifically equal access to the health care system free of charge irrespective of social status and insurance coverage. Additionally, all the administrative registries are based upon the ten-digit unique personal identification number (CPR-number) assigned to all Danish residents at birth or upon immigration, which enables cross-linkage of several registries at an individual level and it provides a unique foundation to conduct large cohort studies with minimal loss to follow-up.

In this PhD thesis all data were obtained through cross-linkage of six nationwide registries: The Danish Civil Registration System, The National *Staphylococcus aureus* Database, The National Patient Registry, The Medical Birth Registry (Fertility database), The Danish Registry for Medicinal Products Statistics (Prescription Registry) and The Population's Education Registry.

The Danish Civil Registration System

The Danish Civil Registration System tracks daily changes in vital status (date of birth, date of death) and demographics (age, sex, immigration/emigration). The registry was established in 1968 and no information is deleted from the registry (97).

The National *Staphylococcus aureus* Bacteremia Database

The National *Staphylococcus aureus* Bacteremia Repository and Database was established in 1957 and contains *S. aureus* positive isolates and clinical information for >90% of all *S. aureus* positive blood cultures in Denmark (98). From all regional hospital departments of Clinical Microbiology, one *S. aureus* isolate from each bacteremia episode is sent to Statens Serum Institut, which hosts as the repository and curates the database and furthermore serves as the national reference laboratory. Prior to 1992 the isolates were registered according to sample numbers, thus it is not possible to combine these numbers with information from other registries. Since 2007 genetic information on bacterial isolates (CC-classes and *S. aureus* protein A [spa] type) have been determined and registered. Way of acquisition (hospital acquired versus non-hospital acquired) has also been recorded in this registry.

The National Patient Registry

The National Patient Registry was established in 1978 and comprises data on all hospital admissions and invasive interventions, and from 1995 and onwards, the registry also contains information on visits in the outpatient care setting. Date of admission, date of discharge, diagnoses and procedure codes are recorded in the registry. The exact date of intervention for procedures or surgery is included, Until 1994, diagnosis codes were registered according to the 8th version of the International Classification of Diseases (ICD-8), and by the 10th version (ICD-10) from 1994 and onwards. Reporting is mandatory, and there is a high level of interest in reporting correctly to the registry, since the reimbursements to the hospitals are based on the codes registered at time of discharge (99,100). The registry has been shown to be accurate (100).

The Danish Registry for Medicinal Products Statistics (Prescription Registry)

Information on all prescriptions dispensed in Denmark (including date of distribution) has been registered since 1995 in the Prescription Registry. Data are registered in accordance with the Anatomical Therapeutic Chemical classifications system (ATC) (101). Validation studies have proved the registry to be highly accurate (102).

The Medical Birth Registry (Fertility Database)

The Medical Birth Registry contains clinical data on all women giving birth (live and stillbirth) in Denmark. All pregnant women, irrespective of employment and socioeconomic status, are encouraged to participate in an antenatal governmentally financed program with several routine appointments at the doctor and midwife. Throughout pregnancy, information is collected in a uniform pregnancy chart and selected data from the pregnancy chart is reported to the registry at time of birth through a standardized software program including the personal identification number of the child. It is further possible to identify siblings and the registered father in the registry. The registry was established in 1973 and has been computerized since 1995 (103).

The Population's Education Registry

The Population's Education Registry contains information on the highest completed level of education for all individuals in Denmark. The Danish education institutions provide annual information regarding enrollment, fulfilled levels of education and exams. In 2008 the registry contained information on 96.4% of the Danes aged 15–69 years, and for 85–90% of immigrants (104).

Study design

For all three papers an observational register-based cohort design was chosen, albeit with different methodologies to accommodate specific requirements related to the particular aim of each study.

Study populations and outcomes

In all papers the study population was consecutively included during the following periods: 1996–2011 (Paper I), 2000–2015 (Paper II) and 1992-2012 (Paper III). Individuals emigrating or acquiring SAB prior to study inclusion were excluded. All individuals were followed until outcome of interest (primarily microbiologically verified SAB), emigration, death, or end-of-study-period, whichever came first.

Paper I

In the first paper of this thesis we assessed the association between socioeconomic status (SES) and acquisition of SAB in individuals ≥30 years of age identified 1996–2012. Besides the overall exclusion criteria, we further excluded individuals without educational information and all first-generation immigrants. The study population was stratified into three age-categories (≥30–50, >50–70 and >70) and information on highest attained educational level (in five categories) at study entry was achieved from the Population's Education Registry. We categorized the educational level as described elsewhere (105) and used the highest level of education as our reference: 1) basic school (primary, lower secondary); 2) upper secondary (general secondary, technical secondary; "high-school"); 3) vocational (e.g. electrician or chef); 4) short or medium length higher education (Academy Profession Degree, Professional Bachelor's Degree, University Bachelor's Degree; 2–4 years following upper secondary); 5) Master's Degree or Ph.D. Further, we examined whether the level of SES influenced the risk of subsequent endocarditis (secondary outcome) in patients with SAB. Information on endocarditis was achieved from the National Patient Registry (ICD-10 codes I33 and I38) and defined as a diagnosis of endocarditis within three months from SAB. These codes have recently been validated with a positive predictive value of 82% (106) and up till 90% when patients were hospitalized for over 2 weeks (107).

Paper II

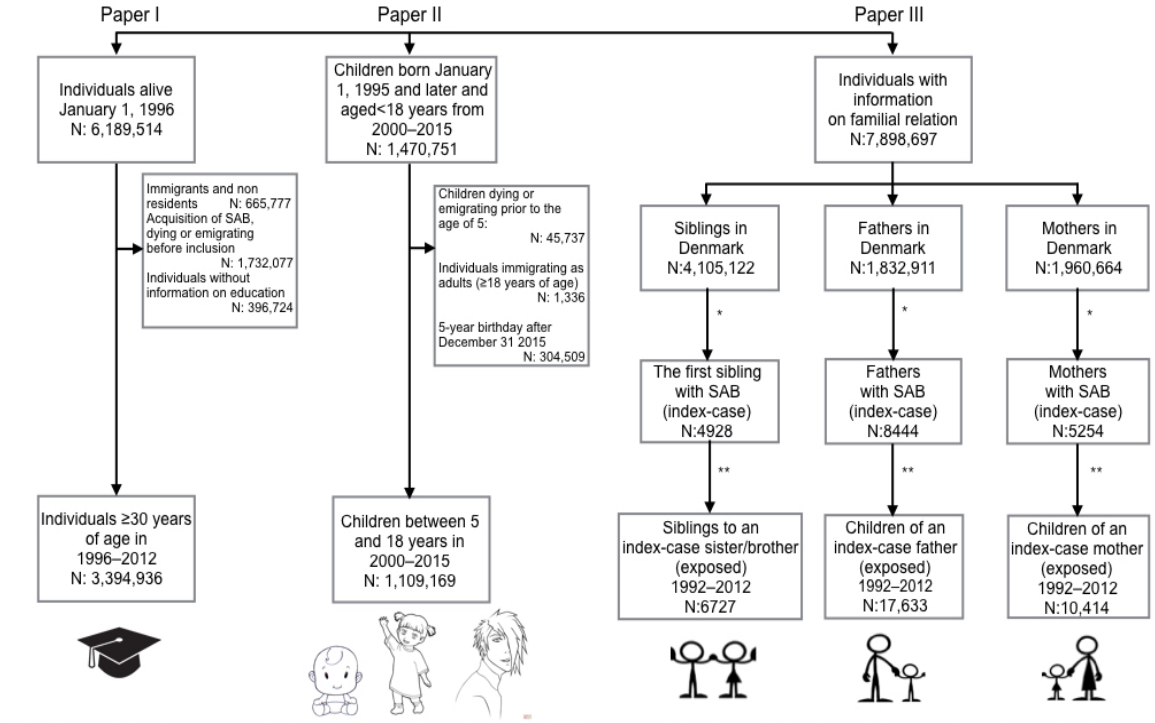
In the second paper we investigated pre-defined risk factors for SAB in a large pediatric population (5–18 years) during 2000–2015 with the child's 18-year birthday as an additional end of follow-up criterium. Cross-linkage of the Civil Registration System, the Medical Birth Registry, the Population's Education Registry, the Danish *Staphylococcus aureus* Database and the National Patient's registry provided us with information on premature birth, acquisition of SAB, comorbidity burden, surgical procedures, and parental socioeconomic status. Children aged 18 years prior to January 1, 2000 or immigrating to Denmark following their 18-year birthday were not included in the study population. The predefined exposures of interest were: Congenital heart disease, chromosome anomalies, cancers, asthma, cystic fibrosis, rheumatic diseases, inflammatory bowel disease, atopic dermatitis, diabetes, burn injury, psychiatric disease with a frequent occurrence of self-mutilating behavior, pacemaker implantation, prosthetic heart valve or an implanted orthopedic device, undergoing transplantation, overall surgery and treatment with dialysis or plasmapheresis (Appendix IV). Among children with SAB we further wished to identify presumably healthy children, and defined presumably healthy children as children without registered comorbidities, who had no contact with the health care system and no claimed prescriptions in the three months prior to SAB and had not undergone invasive interventions in the 30 days prior to the bacteremia.

Paper III

In the third paper, we investigated whether SAB clustered in families with a first-degree relative, who had been hospitalized with SAB using the overall rate of SAB in the general population as our reference. Cross-linkage of the Danish Medical Birth Registry and the Civil Registration System was used to identify and obtain information on all families in Denmark (parents, children, siblings) and through the National SAB Database, data of family members previously hospitalized with a first episode of SAB was achieved. We excluded children without information on their parents, individuals who emigrated prior to study inclusion, stillbirths and adoptees. Families where a parent acquired the bacteremia prior to a child was included in the overall analyses, but not in the sub-analyses.

An overview of the study populations in the three papers and a methodological summary can be found in Figure 2 and Table 1.

Figure 2: Study population flowchart



*SAB= S. aureus bacteremia. *Exclusion of first-degree relatives without SAB: Parents=3 779 877, siblings= 4 100 194. **Exclusion of cases dying or emigrating prior to study inclusion and siblings who were not the first sibling in a sibship to acquire SAB: Siblings=626, children=2513. SAB= S. aureus bacteremia*

Definitions of covariates

The definition and codes used for all covariates were defined the same way in all papers and are summarized in Appendix IV.

Statistical methods

In all papers categorical variables were presented as frequencies with percentages, whereas non-parametric continuous variables were summarized as median with interquartile range (IQR). The median follow-up was calculated as the median time between date of inclusion and first occurrence of SAB (outcome), emigration, and end of study period or death. Crude incidence rates were presented as number of events per 100.000 person-years.

Relative rate analyses

Survival analysis is fundamental to epidemiological studies examining differences in time to the occurrence of a specific event. Since not all individuals in a study population will experience the event of interest during the study's follow-up, their time is censored (right censoring). A crucial assumption is that the process giving rise to right censoring is independent of the pathway to event of interest.

In register-based epidemiological studies both Poisson regression and Cox proportional hazards regression (Cox regression) are widely used survival models to study associations between exposure variables and time to event of interest. Both models calculate rates of event in exposed and un-exposed individuals, and the ratio between rates is a measure of the relative risk.

Poisson and Cox regression models share assumptions on linearity of continuous variables, lack of effect modification (no interactions) and that the ratio of rates is constant over time. However, in Poisson regression the rate ratio is assumed to be constant within each defined time period, thus to evaluate whether the Poisson regression is valid it is essential to test that the results do not change with more narrow time intervals. In Cox regression the hazard rate is calculated for each day since the

beginning of follow-up. The main assumption in Cox analysis is the proportional hazard assumption, that the ratios of hazard for conditions of interest are constant over time.

The main advantage of Poisson regression analyses is that it is possible and technically simple with reasonable computer power to have multiple time-scales and time-dependent covariates in the same model. Since our study cohorts were fairly large and the outcome of interest rare, we chose to use Poisson regression in all papers. To do so we used the Lexis diagram principle (108) to split the observation time in time-bands according to calendar-year and age. Figure 3 displays an example of the Lexis principle, where each line represents the contributing risk time of an individual. In this example four persons are 30 years old at some point in 2006, and due to different length of the lines, they add different risk time to the grey rectangle.

Figure 3: The Lexis diagram

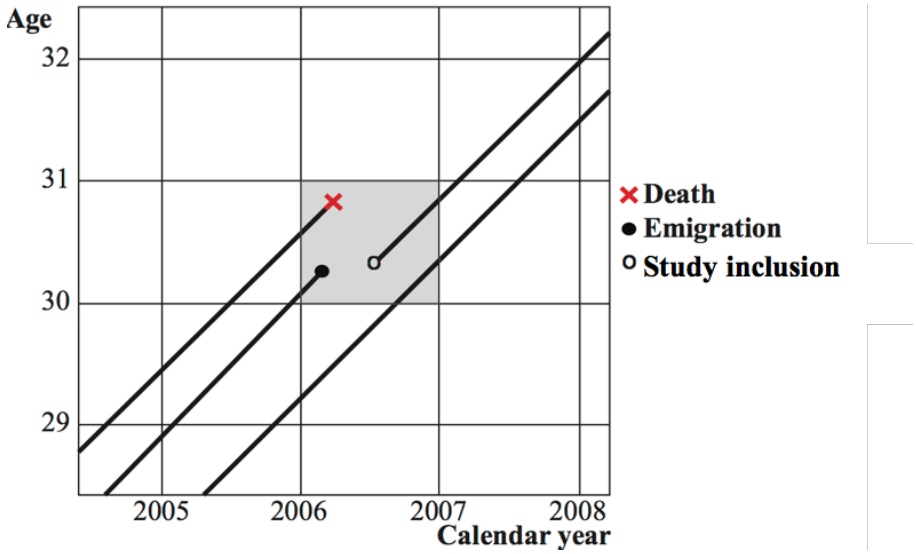


Figure 3: The Lexis diagram visualizes how a given follow-up period is split up according to age and calendar year (2005–2008) and how different individuals (the 4 lines) contribute with different risk time.

Because of the time-dependent approach, we further split the time of follow-up into time-bands according to status of the exposure variables. This enabled each individual to contribute with person-time at risk in the exposed group and in the unexposed group. We further allowed individuals with specific exposure status to switch groups during the study period, e.g. patients requiring treatment with dialysis contributed with risk-time for 90 days following the procedure (exposed to dialysis), and if no other dialysis treatment occurred, these individuals again contributed with risk time in the group of patients without dialysis (unexposed to dialysis).

In the Poisson regression models a rate of event was calculated for each specific combination of calendar year, age and exposure status for all individuals who contributed with person-time to that specific combination.

We reported all estimates with 95% confidence intervals (95% CI) and considered a two-sided p-value below 0.05 as statistical significant. All data management and statistical analyses were conducted in SAS version 9.4 (SAS Institute Inc., Gary, NC, USA). R version 3.4 and Prism 7.0 were used for graphics.

Paper I

In this paper we presented baseline characteristics at time of study inclusion and baseline characteristic were compared across socioeconomic levels. Individuals with the highest SES constituted the reference group for the primary and secondary analyses (SAB and subsequent endocarditis in SAB patient, respectively) and had age and calendar years as underlying time-scales, both in one-year time-bands. To ensure that a lack of an association between SES and risk of

subsequent endocarditis in patients with SAB was not due to a fatal outcome among SAB patients with low SES (survival bias), we explored the cumulative incidence of SAB with death as competing risk.

Paper II

The majority of children included in Paper II were 5 years old at study inclusion and were healthy, thus we found it more relevant to look at characteristics for children acquiring SAB at the time of event. The associations between a number of predefined risk factors and SAB were examined in separate models using children without the exposure of interest as the reference group and by splitting the follow-up period into five-year time-bands since 5- year birthday and one-year age-bands. To explore if the rate of SAB was constant over time following exposure, we conducted sensitivity analyses examining the incidence rate ratios of SAB according to time elapsed since exposure. For most exposures we explored rates during and after 5 years of exposure occurrence, but in accordance with previous studies, we limited elapsed time to 90 days following dialysis, and 30 days following surgery.

Paper III

In Paper III we used a different approach with the rate of SAB in the entire Danish population (the background population) serving as our reference. We found it appropriate to describe the exposed study population according to family status (baseline table). Given the novel design of Paper III, the statistical method warrants a more detailed description. Again we chose a population-based observational cohort design, but the rates of SAB in the study cohort were compared with rates in the general Danish population. The first member in a family to acquire SAB was defined as the index-case, and first-degree relatives to index-cases comprised our study cohort (exposed individuals). Thus, the 'comparison group' for the group of exposed was the entire Danish population represented by population incidence rates. The resulting standardized incidence rate (SIR) calculations were based on individual data on the first-degree relatives and the resulting model was, in principle, a standard technique much used in, e.g. cancer incidence studies (26). However, in cancer studies, population rates are typically obtained from national cancer registries, whereas we were able to calculate 'our own' population rates by having access to individual person level data covering the entire population of Denmark. Exposed individuals were only compared with individuals within the same age- and calendar year time band, thus we accounted for the underlying age differences between the background population and the exposed families. Five-year time-bands for both age and calendar year were used.

To accommodate for the risk of a potential familial clustering being due to simple transmission between first-degree relatives, we analyzed the genetic pattern of *S. aureus* for every infected family member (index-cases and exposed cases) by determining the spa type. If the majority of index-cases and exposed cases were infected with the same *S. aureus* spa type it would favor transmission over a possible genetic susceptibility to the microorganism. Further, shared environmental factors could also explain a familial disposition to SAB, thus we additionally assessed the incidence rate ratio of the disease among spouses. All registered partnerships in the entire Danish population were identified through the Civil Registration System and updated annually, thus not only married couple but all cohabiting couples were included in the variable "spouses". The risk time began the day one of the individuals in a partnership acquired SAB. A high IRR of SAB among spouses would suggest a significant influence of shared environmental factors on a familial clustering of SAB.

Ethical aspects

Danish Data Protection Agency (reference number 2007-58-015/GEH-2014-018 I-Suite number_02736) and the Danish Health and Medicines Authority (reference number 2007-54-0295) all approved the studies included in the current thesis. A specific approval was achieved from the National Research Department at Statens Serum Institut regarding Paper III to obtain information on the spa type *S. aureus* isolates.

In Denmark, conduction of retrospective registry-based studies does not require ethical approval. All data used in the current thesis was held by Statistics Denmark, which additionally had the administrative rights to the data.

Table 1: Methodological summary

	Paper I	**Paper II**	**Paper III**
Aims	To examine the association between socioeconomic status and 1) SAB and 2) subsequent IE	To examine the association between predefined exposures and SAB in children	To examine the associations between a family history of SAB and risk of SAB
Study design	Registry-based nationwide cohort study	Register-based nationwide cohort study	Register-based nationwide cohort study
Study population	The Danish general population ≥30 years between 1996–2012 with information on education	All Danish children from their fifth birthday between 2000–2015	All Danes with information on familiar relations between 1992 and 2012
Stratification	Age-categories: ○ 30–50 years ○ >50–70 years ○ >70 years	Age-categories: ○ ≤11 years ○ >11 years	Stratified in hospital acquired/non-hospital acquired SAB
Exposure	Socioeconomic status	Congenital heart disease, chromosome anomalies, cancers, asthma, rheumatic diseases, atopic dermatitis, transplantation, overall surgery, dialysis or plasmapheresis	Having a first-degree relative previously admitted to a hospital with SAB
Exclusion criteria	Prior diagnosis of SAB, first generation immigrants, or emigration prior to study inclusion	Prior SAB diagnosis, emigration prior to study inclusion, immigration as an adult (≥18 years of age)	**For primary analysis**: Emigration prior to study inclusion **For analyses on family status**: Children acquiring SAB prior to a parent
Outcomes	**Primary**: Acquisition of microbiologically verified SAB **Secondary**: Infective endocarditis (IE) in patients with SAB	Acquisition of microbiologically verified SAB	Acquisition of microbiologically verified SAB
Start of risk time	January 1, 1996 or date of 30-year birthday	The date of 5-year birthday, or immigration	Day of index-case SAB
Follow-up	**Primary**: Until SAB, emigration, December 31, 2011 or death **Secondary**: Until a diagnosis of IE, 90 days after SAB diagnosis, emigration, December 31, 2011 or death	**Primary**: Until 18 year birthday, SAB, emigration, December 31, 2015 or death **Secondary**: Until time since exposure had elapsed 30 days, 90 days or 5 years or 18-year birthday, SAB, emigration, December 31, 2015 or death	Until SAB, emigration, December 31, 2012 or death
Covariates	Age, sex, calendar year, hypertension, peripheral vascular disease, cerebrovascular disease, hearth failure, acute myocardial infarction, valvular heart disease, diabetes, cancer, mild/severe liver disease, acute/chronic renal failure, chronic obstructive pulmonary disease, atopic dermatitis, psoriasis, rheumatic disorders, HIV, dialysis treatment, prosthetic devices and surgical procedures	Age, sex, calendar year	Age, sex, calendar year, socioeconomic status, comorbidity diabetes, cancer, chronic dialysis treatment, any surgical procedure, and presence of a prosthetic device (pacemaker or prosthetic heart valve, knee, or hip)
Statistical analyses	Multiple Poisson regression analyses, Lexis models and Aalen-Johansen estimator for cumulative incidence with death as competing risk	Multiple Poisson regression analyses and Lexis models.	Multiple Poisson regression analyses and Lexis models

RESULTS

This section provides the main results for the three papers in the thesis. Due to the law on anonymization from Statistics Denmark, we were not allowed to report results with less than three affected individuals, thus these results are reported as <3. Each paper is enclosed in the Appendix (Appendices I–III). The figure numbers are related to the numerical order in the thesis and not in the published papers.

Paper I

"The associations between socioeconomic status and risk of Staphylococcus aureus bacteremia and subsequent endocarditis – a Danish nationwide cohort study"

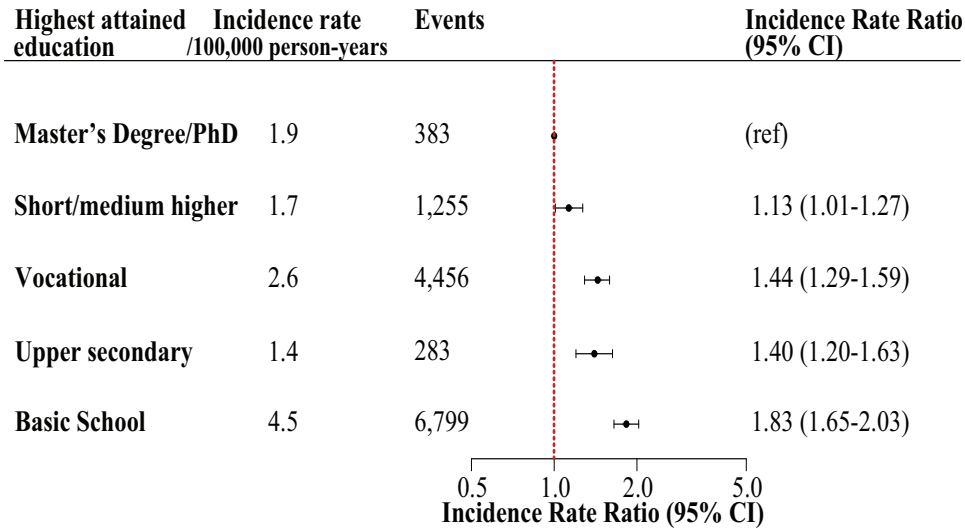
We included 3,394,936 individuals (50.7% male) and followed them from 1996 to 2012 for a median of 15.9 years (IQR = [10.7;16.0]). We observed individuals with low socioeconomic status (SES) to be older with a higher comorbidity burden than the other SES groups. During the study period we identified 13,181 persons with a first hospitalization for SAB (62.9% male). Overall, we observed low SES to be associated with a nearly 2-fold increased risk of SAB compared with the highest SES group and adjusted for age, calendar year and sex, Figure 4.

When stratified by age-categories (p for interaction between age and SES <0.0001), the lowest absolute risk of SAB was observed among the youngest group aged 30–50 years (overall IR=1.1/100,000 person-years) and the highest absolute risk among the eldest (IR=7.7/100,000 person-years for individuals >70 years). In contrast, the highest relative risk of SAB was observed in the youngest group, and the association was attenuated with advancing age, Figure 5. In all analyses stratified by age, no association was observed between having a short/medium length higher education and SAB compared with having the highest educational level (Master's Degree/Ph.D.). The inverse association between decreasing SES and increasing risk of SAB persisted when adjusting for medical conditions, devices and invasive procedures, albeit the association was attenuated (Figure 5 in the paper).

In the post hoc analysis of subsequent endocarditis risk, 776 (5.9% of 13,181 patients) patients acquired endocarditis following SAB (IR of 13.1/100,000 person-years). For the majority of patients (73%, n=569), endocarditis was diagnosed within the first week after hospitalization with SAB, albeit no association between the level of SES and risk of subsequent endocarditis was observed, Figure 6.

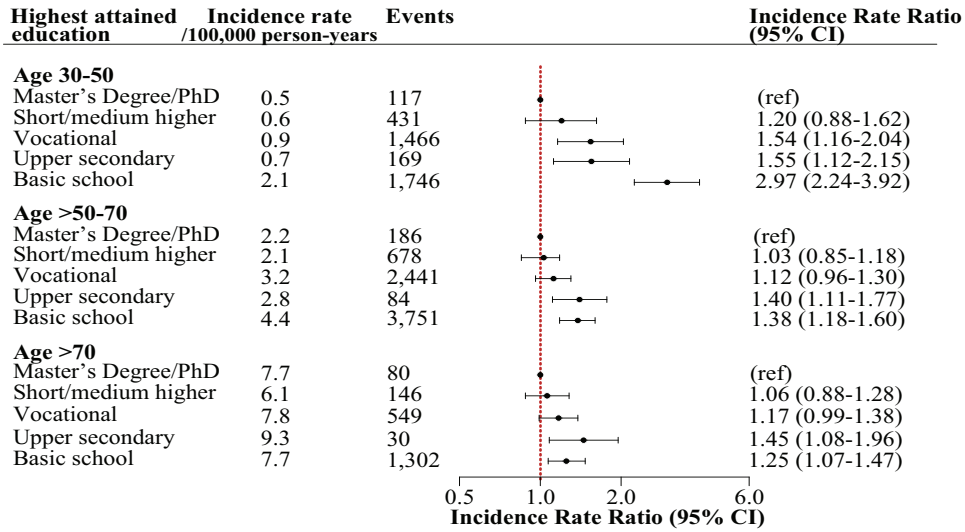
The lack of an association between educational level and subsequent endocarditis in SAB patients was not explained by a higher mortality rate among individuals with low SES, since taking competing risk of death into account did not change our findings (Figure 6 in the paper).

Figure 4: Incidence rate ratios of SAB according to socioeconomic status



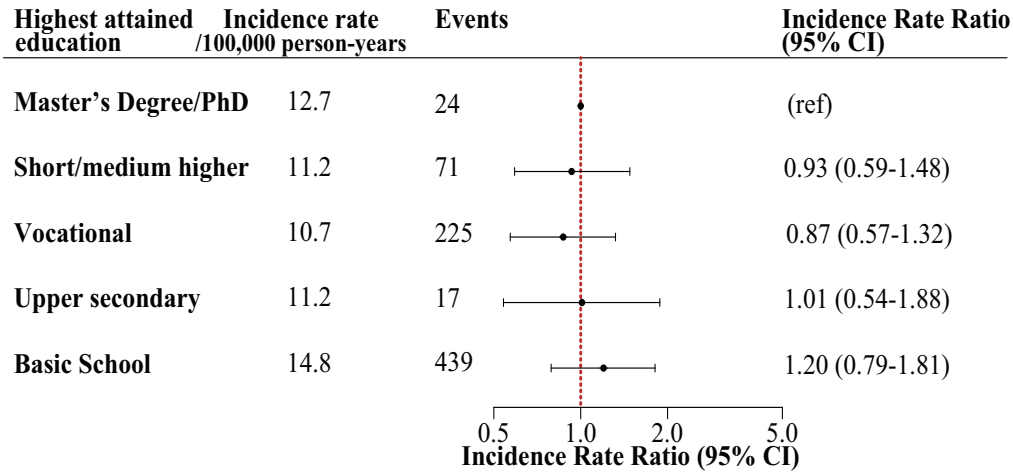
The model is adjusted for age, sex and calendar year.

Figure 5: Incidence rate ratios of SAB according to socioeconomic status and stratified by age-groups



The model is adjusted for calendar year, sex, hypertension, peripheral vascular disease, cerebrovascular disease, heart failure, acute myocardial infarction, valvular disease, diabetes, cancer, mild/severe liver disease, acute/chronic renal failure, chronic obstructive pulmonary disease, atopic dermatitis, psoriasis, rheumatic disorders, HIV, dialysis treatment, prosthetic devices and surgical procedures (highest SES as reference)

Figure 6: Incidence rate ratios of subsequent endocarditis in SAB patients



The model is adjusted for sex, age and calendar year

Paper II

"Staphylococcus aureus Bacteremia in Children Aged 5-18 Years – Risk Factors in the New Millennium"

From 2000–2015 we followed 1,109,169 children (5–18 years of age) and observed 307 children with a first episode of SAB corresponding to an incidence rate of 3.7/100,000 person-years. The incidence of SAB was highest in boys, Figure 7, and 2.6% of the children with SAB died within a year.

Overall, the absolute rates were low except in children with cancer, in transplanted children and in children undergoing dialysis or plasmapheresis, Figure 8. The association between comorbidity and rate of SAB did not differ by sex (all p-values for interaction>0.10), however we observed a significant interaction between age, and cancer, atopic dermatitis, asthma and surgery, and analyses were therefore stratified by age.

We observed several conditions to be associated with an increased rate of SAB, but due to few events (<5), we did not perform relative rates analyses for inflammatory bowel disease, diabetes, cystic fibrosis, burn injury, psychiatric disease, children with a prosthetic heart valve, pacemaker or orthopedic device.

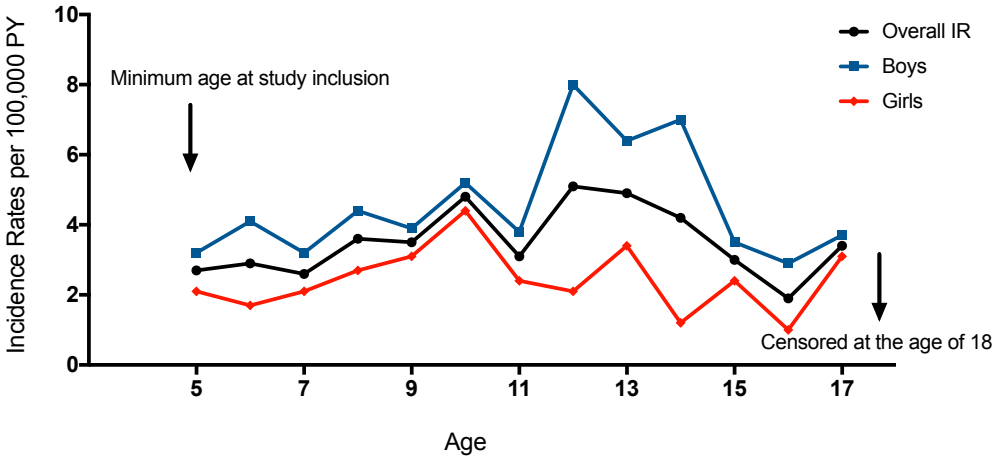
Adjusting for age, sex and calendar year exposed the highest relative rates in children receiving dialysis or plasmapheresis, in children undergoing organ transplantation and in children with cancer, Figure 8. Having a congenital heart disease, chromosome anomalies or a rheumatic disease was associated with an up to 7-fold increased rate of SAB. In children with atopic dermatitis the highest IRRs were observed in older children (≥11 years, Figure 8), and in analyses accounting for time since exposure, the highest IRRs was observed in children ≥11 years within the first 5 years from diagnosis, Figure 9B and 9C. The highest estimates in children suffering from cancer, asthma and in children undergoing surgery were observed in younger children (<11 years of age, Figure 8), and within the first 5 years of cancer or asthma diagnoses and within the first 30 days post-surgery, Figure 9B and 9C.

Examining rates according to time since exposure revealed a significant decline in IRRs of SAB for congenital heart disease, rheumatic diseases and transplantation when time since exposure elapsed more than 5 years, Figure 9A. Conversely, in children with chromosome anomalies, the rate of the infection was increased 1.5-fold following 5 years from diagnosis. In children undergoing dialysis or plasmapheresis, all events occurred within the first 90 days after procedure, Figure 9A. In sub-analyses neither prematurity nor parental socioeconomic status predisposed to an increased rate of SAB in the children.

Among children with SAB 37.1% (N=114) were presumably healthy prior to admission (Table 2) and of these, boys comprised 62.3%. We observed no differences in age at SAB acquisition was observed between boys and girls (age of boys=10.8 [IQR:7.3-14.9], age of girls=10.1 [IQR:8.7-11.5], p=0.7427). Overall, in 2.9% (N=9) of the infected children, soft tissue infection was detected at the time of SAB, however was present in 6.1% (N=7) of presumably healthy children. None of the children with a first episode of SAB had a co-infected sibling at the time of hospitalization (Table 2). Bone and joint infection during admission with SAB was observed in 26.4% of SAB children overall and in 37.7% (N=43) of presumably healthy children. In total, seven (2.3%) children were readmitted with SAB within the first year following primary bacteremia of whom three children had cancer.

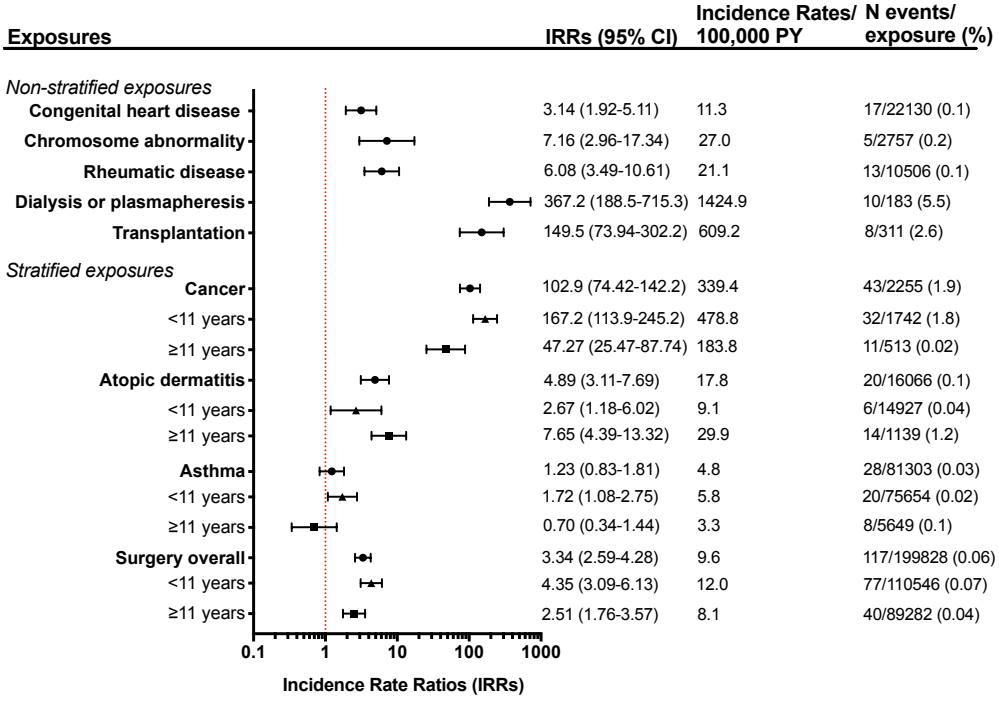
The majority of children with SAB had parents with long educations (47% had a university education; missing values N=3).

Figure 7: Overall Incidence Rate (IR) of SAB in Danish children aged 5–18 years and stratified by sex



Overall incidence rate (IR) of S aureus bacteremia in 1 109 169 Danish children between 2000 and 2015 stratified by sex. PY, person-years.

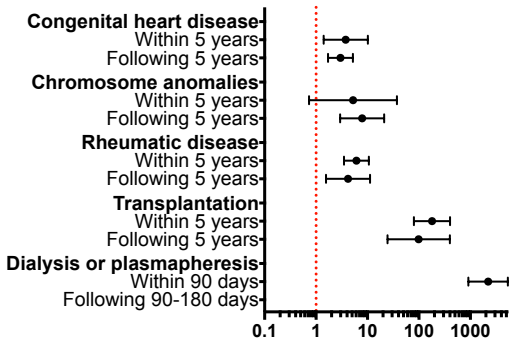
Figure 8: Incidence rate ratios (IRRs) of SAB according to exposures of interest



IRRs of S aureus bacteremia according to exposure of interest in 1 109 169 Danish children aged 5-18 years between 2000 and 2015. All exposure variables were analyzed in separate models and adjusted for age, sex, and calendar year with the reference for each variable being children without the specific condition. N/A, not applicable (too few or no events).

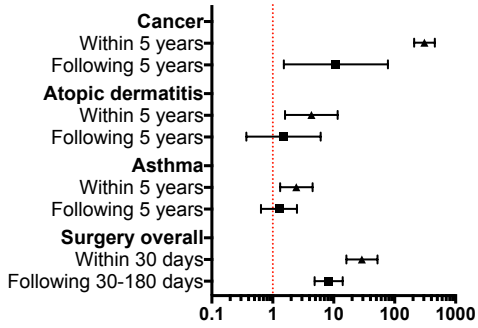
Figure 9: Incidence rate ratios of SAB according to time since the date of exposure

Figure 9A: Non-stratified exposures



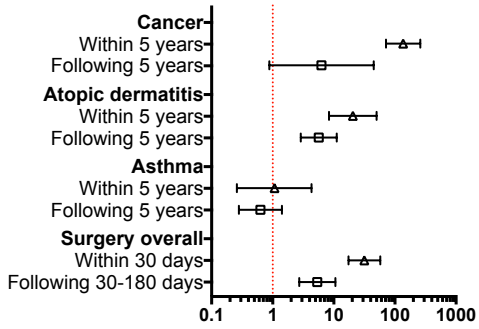
	IRRs (95%)	IR/100,000 PY
Congenital heart disease		
Within 5 years	3.78 (1.41-10.15)	12.9
Following 5 years	2.98 (1.71-5.19)	10.9
Chromosome anomalies		
Within 5 years	5.23 (0.73-37.26)	19.6
Following 5 years	7.89 (2.94-21.16)	29.8
Rheumatic disease		
Within 5 years	6.08 (3.49-10.61)	28.1
Following 5 years	4.19 (1.56-11.26)	16.6
Transplantation		
Within 5 years	180 (80.16-404.0)	714.3
Following 5 years	98.91 (24.55-398.5)	422.9
Dialysis or plasmapheresis		
Within 90 days	2215 (914.8-5363)	4672.8
Following 90-180 days	N/A	N/A

Figure 9B: Stratified exposures (<11 years)



	IRRs (95%)	IR/100,000 PY
Cancer		
Within 5 years	308.5 (209.0-455.3)	844.2
Following 5 years	10.85 (1.52-77.66)	33.2
Atopic dermatitis		
Within 5 years	4.31 (1.59-11.63)	13.5
Following 5 years	1.27 (0.64-2.49)	5.5
Asthma		
Within 5 years	2.44 (1.32-4.51)	7.5
Following 5 years	1.27 (0.64-2.49)	4.6
Surgery overall		
Within 30 days	29.06 (16.13-52.35)	85.7
Following 30-180 days	8.25 (4.85-14.04)	24.4

Figure 9C: Stratified exposures (≥11 years)



	IRRs (95%)	IR/100,000 PY
Cancer		
Within 5 years	136.4 (71.46-260.3)	519.5
Following 5 years	6.28 (0.88-44.97)	24.6
Atopic dermatitis		
Within 5 years	20.47 (8.36-50.13)	75.8
Following 5 years	5.67 (2.88-11.17)	22.4
Asthma		
Within 5 years	1.07 (0.26-4.33)	4.8
Following 5 years	0.63 (0.28-1.42)	2.9
Surgery overall		
Within 30 days	31.66 (17.43-57.50)	113.8
Following 30-180 days	5.37 (2.72-10.60)	19.3

IRRs of S aureus bacteremia in Danish children aged 5-18 years between 2000 and 2015 for specific exposures of interest according to time since date of exposure. All exposures were analyzed in separate models and adjusted for age, sex, and calendar year with the reference for each variable being children without the medical conditions. Because of significant interaction between the exposure and age (P < .05), separate subgroup analyses were performed in children <11 and 11 years of age for cancer, atopic dermatitis, asthma, and surgery. N/A, not applicable (too few or no events).

Table 2: Characteristics of children hospitalized with a first episode of SAB

CHARACTERISTICS OF CHILDREN ACQUIRING SAB IN THE AGE OF 5–18 YEARS	
N	307
Boys (%)	197 (64.2)
Ethnicity (%)	
Ethnic Danes	333 (87.6)
Immigrants	9 (2.4)
Descendants	38 (10)
Not registered	4 (1.0)
Median age at diagnosis (IQR)	10.6 (8.1-13.4)
The most frequent CC-classes since 2007 (%)	
CC45	53 (17.3)
CC30	47 (15.3)
CC15	20 (6.5)
CC1	19 (6.2)
Methicillin resistant *S. aureus* (MRSA)	8 (2.6)
Healthcare contact 90 days prior to SAB (%)	104 (33.9)
Soft tissue infection at SAB diagnosis[1]	9 (2.9)
Complicated SAB[2]	81 (26.4)
Readmission within 1 year from primary SAB (%)	7 (2.3)
Co-infected sibling at the time of SAB	–
Medical conditions (%)	
Congenital heart disease	17 (5.5)
Chromosome abnormalities	5 (1.6)
Cancer	44 (14.3)
Atopic dermatitis	20 (6.5)
Asthma	28 (9.1)
Influenza at admission	–
Cystic fibrosis	<3 (<1.0)
Rheumatic disease	13 (4.2)
Diabetes	<3 (<1.0)
Inflammatory bowel disease[3]	3 (1.0)
Psychiatric disease[4]	3 (1.0)
Burn injury[5]	–
Devices and invasive procedures (%)	
Pacemaker	<3 (<1.0)

Prosthetic heart valve	3 (1.0)
Orthopedic devices[6]	4 (1.3)
Transplantation (solid organ or bone marrow)	8 (2.6)
Undergoing surgery 30 days prior to SAB (%)	53 (17.3)
Dialysis or plasmapheresis	10 (3.3)
Presumably healthy children[7] (%)	114 (37.1)

SAB= S. aureus bacteremia

[1]Soft tissue infection comprised cellulitis, impetigo, superficial abscesses and bursitis. [2]Complicated SAB was defined as SAB with secondary bone or joint foci. [3]Inflammatory bowel disease: Mb. Crohn and Colitis ulcerosa. [4]Psychiatric: personality disorder, anorexia, bulimia, anxiety, affective mental disorder, schizophrenia or psychosis (not drug-induced) [5]Burn injury: Burn injury requiring contact to the emergency department or hospitalization. [6]Orthopedic devices: Device material due to treatment of fractures and hip implants. [7]Previously healthy children were defined as children without comorbidities, without contact to the health care system and no claimed prescriptions three months prior to SAB, and had not undergone invasive interventions in the 30 days prior to the bacteremia

Paper III

"Familial Clustering of Staphylococcus aureus Bacteremia in First-degree Relatives: A Danish Nationwide Cohort Study"

We included 34,774 first-degree relatives (exposed) to 18,626 individuals previously hospitalized with SAB (index-cases) between 1992 and 2011.

In general the study cohort was young and the comorbidity burden at baseline was fairly low. Only few of the first-degree relatives received dialysis treatment or had implanted devices, Table 3.

During the study period 65 first-degree relatives were diagnosed with a first episode of SAB (infected–exposed cohort) corresponding to an incidence rate of 20.8/100,000 person years.

Overall, having a first-degree relative with a history of SAB was associated with a more than 2-fold increased standardized incidence ratio (SIR) of SAB compared with the Danish background population, Figure 10. The rate was particularly high when the index-case had been admitted to hospital with a non-HA bacteremia and when the index-case was a sibling, Figure 10.

Although limited by few events, analyses stratified by sex of the index-case revealed the highest SIRs of SAB in exposed individuals whose index-case was a brother with a non-HA infection, Figure 11. Further, we observed significantly higher estimates among exposed to a male index-case with non-HA infection (brother or father), whereas no significant differences by sex were observed in parents or siblings (p for interaction = 0.85 and 0.92, respectively).

Adjustment for diabetes, cancer, dialysis treatment, implanted foreign material (prosthetic heart valve, pacemaker, or hip or knee prosthesis), and any surgery prior to study inclusion or during follow-up attenuated the results, albeit the associations remained significant with the highest SIR of disease still observed in siblings and in individuals whose first-degree relative had been hospitalized with non-HA bacteremia.

Since none in the infected–exposed cohort had inflammatory bowel disease or rheumatoid arthritis at baseline or during follow-up, adjustment for these conditions was not feasible. Within the families the causative *S. aureus* strain had identical CC-type in only 6 index-case patients and their corresponding first-degree relative (6 from the infected–exposed group), equivalent to 18.5% of the 65 families with an infected–exposed member. Of these, four index-cases and their related cases (67%) were twins less than 1 year of age. We found CC45, CC30, and CC15 to be the most frequent clonal lineages represented in the 65 families, which corresponded to the most prevalent clonal lineages in the Danish Staphylococcal Bacteremia Database (CC45, 20%; CC30, 15%; and CC15, 11%).

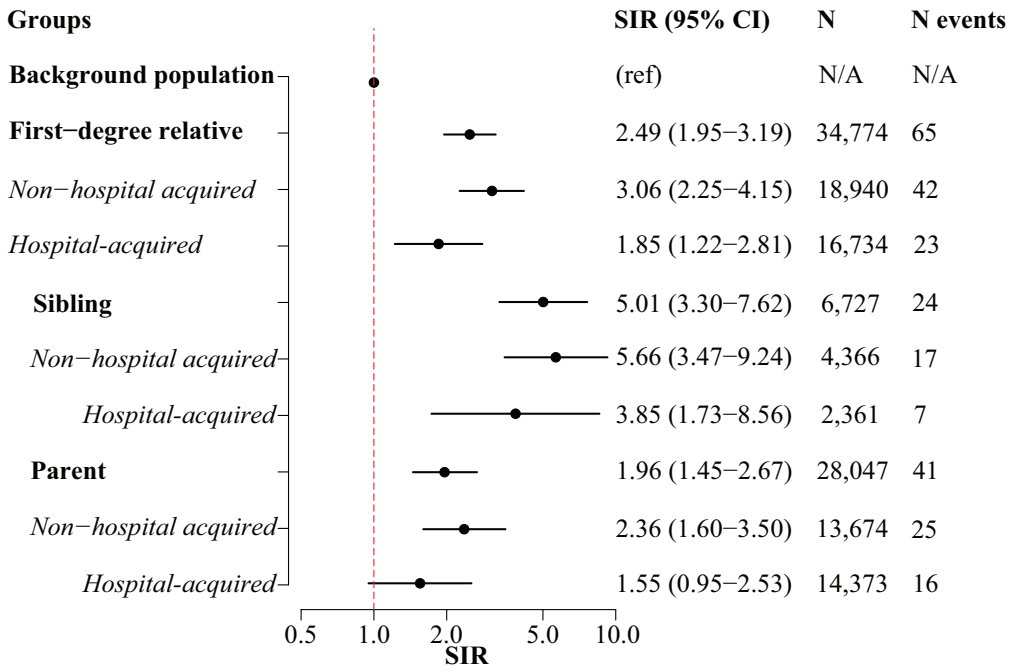
In 57% of the exposed individuals, more than three years had elapsed from the date of the index-SAB to the SAB event of the exposed person. Introduction of a 1-year quarantine period after the index event did not alter our primary findings, albeit estimates were slightly attenuated. We observed no increased risk in spouses to individuals with SAB (SIR, 0.82 [CI, 0.54 to 1.25]).

Table 3: Baseline characteristics of first-degree relatives to individuals previously admitted to hospital with SAB

Characteristic	Siblings	Offspring	
		Maternal	Paternal
Persons, *n*	6727	10 414	17 633
Male	3553 (52.8)	5489 (52.6)	9415 (53.9)
Age			
<30 y	3439 (51.1)	3386 (32.5)	5852 (33.2)
30–40 y	1503 (22.3)	3114 (29.9)	5872 (33.3)
41–50 y	1387 (20.6)	3021 (29.0)	4890 (27.8)
51–60 y	377 (5.6)	879 (8.4)	1008 (5.7)
>60 y	21 (0.30)	14 (0.10)	11 (0.1)
Inflammatory bowel disease	36 (0.5)	69 (0.7)	129 (0.7)
Rheumatoid arthritis	14 (0.2)	38 (0.4)	50 (0.3)
Cancer	36 (0.5)	70 (0.7)	126 (0.7)
Diabetes	99 (1.5)	161 (1.5)	279 (1.6)
Renal disease			
Acute renal failure	16 (0.2)	15 (0.1)	24 (0.1)
Chronic renal failure	13 (0.2)	23 (0.2)	20 (0.1)
Glomerular disease	15 (0.2)	24 (0.2)	22 (0.1)
Dialysis†	3 (0.04)	<3 (0.03)	5 (0.03)
Health care system contact ≤30 days prior to inclusion			
Emergency department	<3 (0.04)	<3 (0.03)	8 (0.05)
Outpatient visits	13 (0.2)	26 (0.2)	29 (0.2)
Hospitalized for 12 h	0 (0)	0 (0)	3 (0.02)
Hospitalized for ≥24 h	27 (0.4)	13 (0.1)	25 (0.1)
Devices‡	<3 (<0.04)	5 (0.05)	<3 (<0.02)
Surgery§	35 (0.5)	32 (0.3)	76 (0.4)

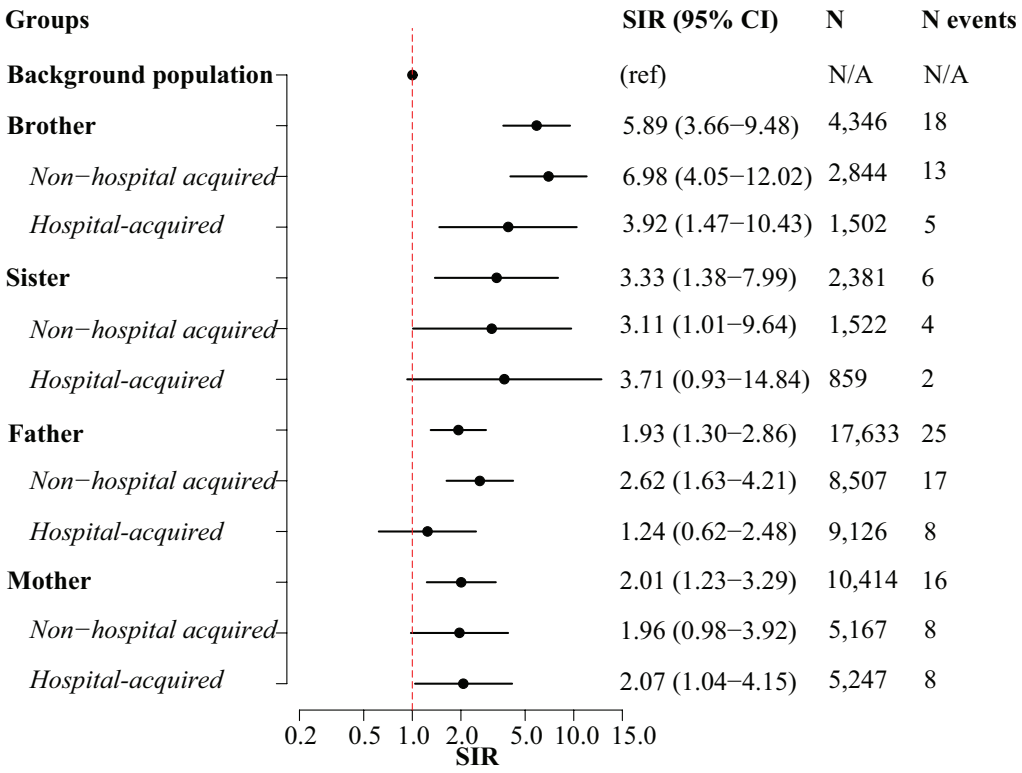
**Values are numbers (percentages) unless otherwise indicated. Percentages may not sum to 100 due to rounding. † Dialysis treatment regardless of indication within 6 months prior to study inclusion. ‡ Implantation of a pacemaker (regardless of type), prosthetic heart valve, or hip or knee prosthesis within 1 year prior to study inclusion. § Any type of surgery within 1 month prior to inclusion.*

Figure 10: Standardized incidence ratios of SAB for exposed overall, by family status of the index-case and by way of bacteremic acquisition of the index-case



The model is adjusted for sex, age, and calendar year. NA = not applicable; SIR =standardized incidence ratio.

Figure 11: Standardized incidence ratios for SAB in first-degree relatives stratified by sex, method of bacteremic acquisition, and family status of the index-case patient



The model is adjusted for sex, age, and calendar year. NA = not applicable; SIR = standardized incidence ratio.

DISCUSSION

SAB is a rare disease in otherwise healthy individuals as confirmed by the low absolute risks in all three papers constituting this thesis. As a result hereof, even a few extra events in one exposure group would lead to a high relative risk, e.g. a 2-fold increased risk could be from 1/10 to 2/10, and 1/1,000,000 to 2/1,000,000. Importantly, the main focus in this thesis was not the absolute risks of SAB, but rather an understanding of factors influencing the acquisition of the disease, e.g., the association with low SES particularly in young adults, that a significant proportion of children acquiring SAB was previously healthy, and that genetics *may* have a role since this rare disease appeared to cluster in families.

Paper I

Main findings

In an adult population above 30 years of age we observed the level of SES to be inversely associated with SAB particularly in adults between ≥30–50 years of age. SES was not associated with subsequent infective endocarditis (IE) in patients with the bacteremia and the lack of an association was not explained by a higher mortality rate in SAB patients of lower SES compared with SAB patients of high SES.

Comparison to other studies in the field

Various measures have been used to reflect SES across studies (e.g. income, education, crowding, low income areas), nevertheless the literature indicate that low SES increases the general risk of infectious diseases (19,27,28,90,109–112). In New Zealand and Australia severe infectious diseases including SAB are a major cause of hospitalizations with strong inequality in SES and ethnicity (19,26,90). In both countries the rate of SAB is markedly higher among individuals of lower SES and with the Pacific Islanders, Maoris and the indigenous people in Australia being the most vulnerable compared with European and non-indigenous people, respectively. An index score constructed from various variables e.g. educational level, occupation type, employment status, income housing status and single parenthood status was used to define SES in these studies.

In a Danish case-control study exploring the association between SES and community-acquired bacteremia in working-age people SES was measured according to both income and educational level. The researchers stated that in contrast to income, educational level may be a better marker of SES when people are young, whereas income may reflect SES better later in life. For both markers a low level was associated with an increased risk of bacteremia and the substantially higher burden of alcohol and drug abuse among individuals with low SES explained close to 50% of the differences in bacteremia rate across SES levels (27). Studies have found that an increased susceptibility to infections in people of lower SES could be explained by poorer hygienic practices, household crowding, more smoking, poorer nutrition and less use of vaccines (90). In countries without a universal healthcare system inequalities in access to hospitals and professional healthcare treatment are probably explained by differences in the ability to finance sufficient health insurance and could also explain a longer patient delay in individuals from the most deprived social class. However, in Denmark the access to the healthcare system and medical treatment is equal and free of charge irrespective of SES.

The literature further suggests variations in comorbidity burden as a mediator of the significant disparities in the rate of infectious diseases, including SAB, across SES levels. Several chronic diseases have been observed to be more prevalent among individuals of low SES e.g. diabetes (48,50), liver disease (56), cancer (113), human immunodeficiency virus infection (59,114) and conditions related to substance abuse, which are all associated with increased risk of SAB. However, adjustment for several chronic diseases only attenuated our results.

Although SAB is a prominent cause of IE worldwide, and complications to SAB frequently occur in individuals of low social status (27,115), we are not familiar with other studies examining the association between SES and the risk of subsequent infective endocarditis (IE) in patients with SAB.

Methodological considerations

To examine the association between SES and risks of SAB and subsequent IE we followed all Danish citizens ≥30 years of age. The lower age limitation was chosen to make sure that the majority of the population had finished their education. In un-published analyses we tried to set the age-limit at 25 and 35, which did not change the results significantly.

The association between SES and acquisition of SAB differed by age (p for interaction <0.0001), thus we stratified the population into three age groups: 1) 30–50 years of age, 2) >50–70 years of age and 3) >70 years of age. In Denmark the official age of retirement is 65 years, however it is becoming increasingly common for the elderly to keep working after the age of 65 years, thus the higher threshold was chosen to ensure that patients in this age group were in fact retired. Changing the age-categories in a supplemental analysis revealed that the increased estimates were still driven by the increased risk in the youngest subjects, which supports our age-categorization.

In our study, we used educational level as a marker for SES, which is unlikely to change markedly after early adulthood and more important it is a strong predictor of an individual's future occupation and thereby also future income (116). In a Danish case-control study the researchers chose to only include individuals in the working-age arguing that educational level and income is a less reliable measure of SES in the elderly (27,116). An ideal way of defining socioeconomic status in either extremes of life does not exist. Despite this limitation, we were interested in examining if the level of SES was associated with the risk of acquiring SAB throughout adulthood. Since educational level is known to reflect income later in life (116), we chose to define SES in accordance with highest attained educational level at study inclusion. If SES had been defined by income a number of individuals would have been categorized as having a lower SES: 1) Students who are given subsistence throughout their studies, i.e. particularly individuals with the longest education would be misclassified, 2) newly educated individuals who receive a lower salary after end of their studies (e.g. doctors) and 3) retired individuals who receive pension. However, our results did not change when income was used as socioeconomic marker in a supplemental analysis.

Lastly, a number of studies have observed SAB to be more frequent among different ethnic groups (e.g. Indigenous people, Pacific-Islanders, Maoris, African-American) and further suggest that the higher rate of *S. aureus* infections and SAB among people of lower SES could be mediated by ethnicity (19,28,90,111,113,117). We chose not to stratify our analyses by ethnicity since 1) ethnic Danes constituted approximately 90% of the Danish population during the study period, thus the variety in ethnicity is minor compared to other countries in the world (e.g. the United States, Australia and New Zealand), 2) we chose to exclude immigrants since the Population's Education Registry does not include educational information on educations fulfilled in the home country of immigrants. 3) Differences in susceptibility to *S. aureus* among diverse ethnic populations was not only explained by a higher comorbidity burden and more deprived household living among individuals with low SES but also on access inequality to the healthcare system. Denmark has a unique universal healthcare system with equal and free access for all citizens irrespective of SES, thus we believe that differences in ethnicity regarding SAB risk would only influence our results a little.

Novelty

This paper is the first nationwide cohort-study exploring the association between not only the level of socioeconomic status and acquisition of SAB but also whether SES is associated with the risk of subsequent endocarditis in SAB patients. Since the associations were strongest in the youngest (30–50 years), these results suggest that the physician should pay extra attention to younger individuals of low socioeconomic status, particularly when they present with diffuse symptoms.

Paper II

Main findings

Paper II explored potential risk factors for SAB in a nationwide pedatric poplution between 5 and 18 years of age. SAB was uncommon in children above five years, Factors prone to increase the risk of SAB in adults (dialysis, plasmapheresis, receiving organ transplantation and having cancer) were associated with the highest relative rates in children aged 5–18 years. More than a third of the

infected children were presumably healthy, emphasizing the need for awareness of the disease also in children without identified risk factors. Premature birth and parental socioeconomic status did not increase the disease rate as observed in studies including younger children.

Comparison to other studies in the field

Overall our main findings were in line with published studies of selected and smaller children populations and studies of adults. Dialysis, plasmapheresis, surgery and organ transplantation were all strongly associated with SAB in our child cohort, as observed in other pediatric studies (39,66,118). One study including 68 children receiving dialysis found that 33% of the pathogen cultured were coagulase-positive and of these 72% were *S. aureus* (39). Another study identified 38 *S. aureus* infections among 698 transplanted children of which 15.3% were bacteremia. The majority of *S. aureus* infections (90.2%) occurred more than six months after the transplantation, and heart transplantation was associated with the highest risk of bacteremia (66).

Further, we observed congenital heart disease, rheumatic diseases and chromosome anomalies to be associated with acquisition of SAB. Congenital heart disease is often diagnosed within the first year of life in particularly if the disease confers hemodynamic problems. Moreover, in these children surgery is often necessary, and a combination of invasive procedures, long hospitalizations in children with an age-related immature immune system markedly increases the infection risk (119). In our child cohort, congenital heart disease increased the incidence rate of the bacteremia more than 3-fold, which was probably explained by late diagnosis in some of the children and surgery later in life due to a less severe heart disease.

Having a rheumatic disease was associated with more than a 6-fold increased rate of SAB in our child cohort within the first 5 years after diagnosis. A study from Finland observed juvenile idiopathic arthritis to be associated with a 3-fold increased risk of bacteremia, whereof *S. aureus* was a frequently observed pathogen (44). Additionally, a study from the United States found an increased rate of infections requiring hospitalization especially among children treated with glucocorticoids, which has also been confirmed in adults (121). Whether it is the inflammatory aspect of the disorder or the immunosuppressive medication used to stagnate the disease, which confers the increased risk of SAB is not fully elucidated.

Moreover, our study might be the first population based study to reveal an increased rate of SAB in children with Trisomi 21 (Down's Syndrome). Although, studies on SAB in children with chromosome anomalies are rare, a study from the United States including 26 patients with Down's Syndrome observed a high occurrence of SAB in these children (122). Additionally, children with Down's Syndrome suffer from a compromised immune system, often have an increased rate of *S. aureus* skin infections and a high comorbidity burden (122,123), thus have an increased risk of SAB. Further, a study from Oxford revealed a 19-fold increased rate of leukemia and an over 2-fold increased rate of type 1 diabetes in children with Down's Syndrome, medical conditions known to increase the risk of SAB compared with children without the disease (124).

In line with other studies conducted in children and adults (32,34,118) cancer was strongly associated with SAB. The doubled incidence rate of SAB in children with cancer <11 years of age, and the marked decline in the relative risk after five years from diagnosis could be explained by more than 80% of Danish children diagnosed with cancer between the age of 0–15 years being alive and cancer-free five years from diagnosis. Further, the majority of these children are not in the need for intravenous accesses prone to increase the infection risk.

Further, atopic dermatitis and asthma were associated with acquisition of SAB. In children with atopic dermatitis the rates of SAB were highest among children above 11 years of age and within the first 5 years after diagnosis. The median time of atopic dermatitis persistence is 3 years from diagnosis. Most children remit from their atopic dermatitis during childhood, but children with severe disease or late onset of the disease have an increased risk of persistence in adultness (125). This is in accordance with our observation that in children with atopic dermatitis the rates of SAB were highest among children above 11 years of age and within the first 5 years after diagnosis. In contrast, asthma was only associated with increased acquisition of SAB in children younger than 11 years of age, which could be because childhood asthma remits in approximately 50% of children before the age of 18 years (126).

Co-infection with influenza at the time of hospitalization with SAB is well-known in children and complications with necrotizing pneumonia due to *S. aureus* confers a major cause of death in children with influenza (127,128). It would have been of great interest to examine the association between influenza and acquisition of SAB, however none of the children acquiring SAB in our study had a diagnosis code of influenza at the time of hospitalization, thus impeding further analyses.

The association between parental socioeconomic status, prematurity and acquisition of SAB was among the variables, which is far from elucidated. A few studies (two Danish and one Swedish study) have shown that low parental educational level, low parental income and living in low medium income areas are associated with an increased risk of hospitalization in children less than 5 years of age (29,30,129). Importantly, children younger than 5 years old have an age-related impaired immune system, which could have influenced the results from these studies. In contrast we observed no association between parental socioeconomic status and SAB in children between 5 and 18 years of age, which may be explained by our inclusion of an older child population with a more robust immune system. Moreover, the lack of an association could be because risk factors increasing the risk of SAB in adults with low SES (e.g. diabetes with complications, manifest cardiovascular disease and intravenous drug abuse) are simply not present in children aged 5–18 years of age.

The older child cohort likely explains why premature birth was not associated with acquisition of SAB in our population as observed in other studies (120,130), indicating that children born prematurely and surviving the first five years without acquiring SAB have a risk of SAB comparable to that of children born at term.

Although the majority of children hospitalized with SAB suffer from comorbidities, our study confirmed that presumably healthy children constitute a significant proportion of children with SAB (25,74,89,118,131,132). In an American study examining 151 children with SAB, 47.4% (N=27) were considered healthy prior to SAB (50% of the children were younger than 1 year of age when acquiring the bacteremia). A British study examined 2,364 consecutive episodes of bloodstream infections in a children's hospital and observed 281 (11.9%) children to be previously healthy prior to the infection (74). Investigators from New Zealand observed significant part of SAB children <16 years to be previously healthy (89). In an Australian study 59% of previously healthy children presented with bone or joint infection prior to SAB (119). In our study we observed bone and joint infections to be more frequent in presumably healthy children compared with comorbid SAB children. These findings also underscore that the proportion of previously healthy children in SAB cohorts is highly dependent on patient selection and definition of "healthy".

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Methodological considerations

Studies of risk factors for SAB in the pediatric population are sparse and the current literature is mainly of descriptive character and focuses on infants. Due to the vulnerability in the first years of life, we specifically chose to focus on children aged 5–18 years, thus children who were not challenged by an age-related immature immune system and thinner skin-barrier (133,134). Further, we specifically chose to name previously healthy children as "presumably healthy children", since it was out of the scope of this paper to reveal whether these children were in fact healthy or whether the onset of SAB was a proxy for an underlying disease to be discovered.

Since our population was limited to children aged 5 to 18 years, we were limited by fewer events compared with other studies in the field, since the incidence of SAB are high among infants, and thus we only adjusted for the most important confounders in the statistical models.

We chose to include children born from January 1, 1995 and later even though information regarding microbiologically verified SAB was available before this date. This decision was made since information on prescribed and subsequently claimed medication and outpatient care was not available prior to 1995. The latter is specifically important since children in Denmark are often referred from the general practitioner to outpatient care settings instead of being hospitalized, thus hypothetically we would underestimate exposed children by including years where information on outpatient care was not available.

The definition of dialysis and plasmapheresis conferred further considerations. Dialysis and plasmapheresis are rare in children, but are strongly associated with increased risk of SAB and are two similar procedures conducted through a rather large intravenous catheter. We therefore found it reasonable to combine these procedures in one variable.

Lastly, the main model used in this study anticipated constant rate ratios over time, but assuming that the rate of SAB would be constant regardless of time since exposure would be incorrect. Therefore, in a supplemental analysis we examined the rate of SAB according to time since exposure. Due to a median follow-up of 7.8 years (IQR: 3.6-11.9) a 5-year period was considered appropriate for permanent exposures (e.g. congenital heart disease, diabetes, rheumatic disease). A 90-day period was chosen for children receiving dialysis or plasmapheresis in accordance with the current literature. A 30-day period was chosen for children undergoing surgical interventions due to a general consensus that infections or bleeding within 30 days post-operatively is likely to be a consequence of

the surgical procedure. However, since we did not differentiate between surgical procedures with or without implantation of a foreign body, 90 days may have been more appropriate since studies have revealed an increased risk of infection within the first 90 days after surgery when a foreign body was implanted. Nonetheless, we believe that this is a minor limitation, since implantation of a foreign body in children is rare.

Novelty

To the best of our knowledge Paper II is one of the largest studies in the field of SAB in children and unique in the number of pre-defined exposures explored in a nationwide setting with universal access to the health care system. Further, we wanted to examine potential risk factors for SAB in a pediatric population, which was not influenced by an underlying age-related vulnerability to infectious diseases due to an impaired immune system. Thus, in contrast to other studies, we specifically chose to include children above the age of 5 years since children between the age of 5 and 18 should be able of eradicating an invasive *S. aureus* in contrast to younger children. Also, we are not aware of other studies examining parental SES as a potential risk factor in this case. Besides increasing the awareness of the bacteremia in children with comorbidities, these results also encourage the physician to consider this diagnosis even though a child is presumably healthy (no chronic diseases, no contact with the healthcare system 3 months prior to SAB and no medication 3 months prior to hospitalization with SAB).

Additionally the results pave the way for future pediatric studies to explore sex differences in risk profile especially in the early teens, and to investigate if SAB is a marker of an underlying disease in previously healthy children. If not it would be of great interest to conduct a study in these children to reveal potential genetic differences among healthy children acquiring SAB compared with an age- and sex-matched child cohort without SAB. Importantly, this study should include information on all factors that could potentially increase the risk of SAB such as visits to the dentist and comorbidities treated solely at the general practitioner or by the help of doctors from private clinics, which was not possible in Paper II.

Paper III

Main findings

The main findings were that having a first-degree relative with a first episode of microbiologically verified SAB increased the risk of acquiring the disease compared with the risk in the general population. The observed increase in relative risk of SAB was low compared with risk associated with other risk factors for this bacteremia (e.g. cancer, HIV, catheter placement) but age-related comorbidities were considered to only comprise a minor confounder since we studied a cohort of relatively young and healthy individuals.

Comparison to other studies in the field

Familial aggregation has been observed for other infectious diseases. In a Swedish cross-sectional study on monozygotic and dizygotic twins the authors observed a genetic influence on the susceptibility to H. Pylori. Both twins reared apart and reared together were examined, and shared environmental factors were observed to also influence the susceptibility (135). Further, in a population-based study from the United Kingdom the authors observed an up till 30-fold increased risk of meningococcal disease among siblings compared with the rate of infection in the general population (136). When more than one year had elapsed between the index-case event and the case event, the relative risk declined to an 8-fold increased risk, nevertheless still suggesting that a genetic component could play a role in the susceptibility to *Neisseria meningitidis*. However, the study was based on questionnaire data, which might have been subject to recall bias. Conversely, a Danish study following 43,134 relatives of individuals with invasive pneumococcal disease revealed an increased infection risk among closely related persons (parents, children, siblings and half-siblings), but only when the relative acquired the infection within the first year of the index case. The increased risk was probably caused by shared environmental factors and no increased risk was observed in

third generation family members (grandparents), or when the relatives did not share the same household with the case (137). In contrast, the main findings of our study remained close to unaltered when only events of SAB occurring one year after the index date was explored and when only individuals aged 20 years and older were considered at risk. Further, in most cases, more than three years elapsed from index-case event to case event and >80% of the infected cases were infected with a *S. aureus* strain genetically different from the index-case making direct transmission unlikely to drive these results. Given that no increased rate among spouses was observed, our results did not appear to be driven by shared environmental factors.

If genetic host factors influence the susceptibility to *S. aureus*, it is reasonable to expect that the risk would be attenuated as familiarity becomes more distant. However, information in *The Danish Fertility Registry* about parents to individuals born in Denmark was not complete until 1954 and onwards, which precludes an analysis about risk in grandchildren. The same argument precludes analyzes of the risk of SAB in cousins, since the cousins have to be identified through their parents, thus the cousins possible to identify would be too young for analyses to be reasonable, particularly since SAB most often appears in the elderly.

Host genetics would also be assumed to be mostly relevant to community-acquired SAB, since several non-genetic factors may play a role for acquisition of HA SAB (e.g. indwelling catheters, medical devices and surgery). Interestingly, stratifying the way of acquisition in the index-case into HA and non-HA SAB revealed higher relative rates of bacteremia in cases to non-HA index-cases, and the first-degree relatives to non-HA index-cases were more likely to acquire non-HA SAB themselves. In contrast, in index-cases hospitalized with HA SAB, the distribution of non-HA and HA SAB were equally distributed among the cases. All together, these findings support the hypothesis that genetic host factors play a role in the susceptibility to *S. aureus*.

Several studies have examined the influence of host genetics on the susceptibility to *S. aureus*, where the ability to transfer genetic susceptibility to the microorganism between different mice species is considered a strong indicator that genetic host factors play an important role in the sensitivity to *S. aureus* (77–79). Moreover, a number of studies have examined genetic host factors in humans related to increased infection risk and suggest mutations in several candidate genes such as two genes on chromosome 11 (80), STAT3 (leading to Job's syndrome) (94) and alterations causing IRAK-4 (interleukin-1 receptor-associated kinase 4) deficiency (92). Additionally, mannose-binding lectin deficiency, present in 5–10% of all individuals, impairs the innate immune system, increases the risk of infections with *S. aureus* and influences the outcome of patients with infective endocarditis (82,138). No common variants were observed to increase the risk of SAB in a recent genome-wide association study, however this research did not address low-frequency or rare variants that may be associated with increased susceptibility in humans, and the study was further hampered by a small study size (96). In contrast another recent study observed variations in the gene GLS2 to be associated with complicated SAB.

In contrast in a study using whole exome sequencing (WES) in patients with complicated SAB (endocarditis/bone or joint infections), variants in GLS2 was observed to be associated with complicated SAB. GLS2 regulates the production of glutamine, thus indirectly the activation of T-cell production which is essential for combatting SAB, which makes GLS2 an interesting candidate gene (139).

Methodological considerations

In this paper the study population consisted of first-degree relatives (cases) to individuals previously admitted to a hospital with a first episode of SAB (index-cases). We chose to exclude adopted children, even though these children would appear ideal for elucidating environmental and genetic influence on SAB occurrence. An increased risk in adoptees would suggest that environmental factors or unmeasured confounders rather than genetics would be responsible for a familial clustering of the infection. There are however important limitations to such an analysis, which would make the results difficult to interpret. First, adopted children represents a tiny part of the Danish population, and information on adoptees was only included from 1980 and onwards. Secondly, there is an insufficient registration of the adoptive parents, thus for 38.6% of the adoptees, information on the adoptive parents' identification numbers were missing which prohibited us from obtaining information on SAB. However, we identified 33,626 children, who were registered as adopted in Denmark during our study period of whom 20,525 adopted children had parental information (n excluded due to missing data on parents=12,996). Of these children, 188 adoptees had a first-degree relative with SAB, but none of the adoptees (children/siblings) acquired the disease, thus precluding

any additional analyses.

Another limitation to the study was that approximately 10% of registered fathers are not the biological fathers (140). As a final minor limitation, the father to children conceived through sperm donation would not appear in the registry. Since single mothers often prefer to use the same sperm donor, these children would be detected as half-siblings in the data management process, although they have the same mother and father.

One could argue that a matched case-control study would be simpler for the reader to understand, however we believe that our approach had at least two advantages compared to having an 'internal, unexposed control group' obtained, e.g., by matching 10 unexposed subjects by age, sex, and period to each exposed first-degree relative. First, we used *all the data* from the country on incidence of SAB (and not only data from the ~34.000 exposed and 10*34.000 matched unexposed), which is advantageous for this rare condition. Second, a more subtle advantage is that by comparing the exposed group to the entire population instead of comparing to an "unexposed" group, we avoid a potential (albeit small) misclassification of exposure by not knowing about exposure before 1992. In a matched case-control study we would also know for certain that the ~34.000 exposed are truly exposed but among the matched unexposed there might be few with a first-degree relative diagnosed prior to 1992.

Novelty

We are not familiar with other studies exploring a familial clustering of SAB and the method is the first of its kind, thus Paper III is unique in several aspects. Knowledge on familial clustering can lead the way for future genetic research to clarify whether and to which extend a genetic component is present in the risk profile of SAB and explore the possibility for identifying high-risk groups, and potentially prophylactic actions. Further, the method can be used in many scientific fields to serve as a pilot study prior to large, expensive genetic studies.

Strengths and limitations

Randomized controlled trials are the "Gold standard" in research. However, due to ethical, practical and financial circumstances randomized controlled trials are not always feasible. The studies comprising this thesis were observational and based on historical data obtained from numerous Danish administrative registries, thus none of the variables included in our models were self-reported or achieved from questionnaires, thus *recall bias* was not an issue.

Although the epidemiological approach has limitations, it additionally has several advantages. The studies were conducted in a nationwide setting with a universal healthcare system, which minimized *selection bias* particularly for Paper I and Paper III. Further, the risk of missing data or incorrect reporting was limited since most of the nationwide registries function as governmental administrative registries, and it would therefore have financial consequences if reimbursement were missed. Moreover, the continuous reporting to the registries over decades provided large study cohorts with a minimal loss to follow-up, which is crucial when exploring rare events.

Importantly, SAB was the primary outcome in all papers, information on which was achieved from the National Surveillance Database, thus despite the use of registries, our outcome was microbiologically verified. Further, our primary analyses in all three papers were adjusted for age, sex and calendar year, which are three very robust variables. Particularly important for Paper II, we achieved information on comorbidities and invasive procedures from the National Patient Registry (100). Although a number of validation studies have been published, the majority of validation studies are conducted in the adult population, thus it is not examined if the diagnosis codes registered at discharge in the patient files for children are compliant with the codes in the registry. The majority of validation studies have studied the positive predictive values of the diagnoses defined as the compliance between the discharge diagnosis in the registry, and the discharge diagnosis in the medical record. However, only a few validation studies have studied the true positive predictive values, thus whether the patients actually have the diseases, nor examined the sensitivity of the diagnosis codes, thus whether patients with the disease achieve the correct diagnosis code (99,106,107,141). The discharge diagnoses are assigned by the treating physicians and the results of all register-based studies must therefore always be interpreted in light of the circumstances under which the clinicians contribute to the registries, e.g. the death certificates constituting the National

Causes of Death Registry are often the result of doctors speculating on the causes of death, sometimes without having ever met the patient. The independent variable (educational level, SES) in Paper I was achieved from a valid register where above 96% of education for all individuals between 15 and 69 are recorded (104). Likewise, information on first-degree relatives was achieved by combining The Civil Registration System and the Fertility Register, and although an estimated 10% of Danish children have another biological father than the one registered in the registries the independent variables in Paper III must be accounted robust.

The PhD thesis has limitations, which merit comments. The observational design of the studies made examination and interpretation regarding associations possible whereas further prospective studies are warranted to confirm our results and explore causality. The factors identified as associated with the risk of SAB may thus not be risk factors, but rather markers of unmeasured confounders. Observational studies will almost always be influenced by residual confounding, thus influenced by inadequately or unmeasured factors. In all papers we were not able to investigate if there was regional differences in SAB incidence, obtain information on conditions handled by the general practitioner or immunosuppressive medications not requiring a prescription (e.g. chemotherapy administered at the hospital or particularly expensive drugs e.g. rimactan dispensed from the hospital), identify focus of the infection, or achieve information on nasal carriage of *S. aureus* or drug abuse, which could potentially have influenced our results. Specifically, in Paper I microbiological information about patients with endocarditis was not available to us in any registries, thus all endocarditis events may not have been caused by *S. aureus*. However, the possible overestimation most likely only constituted a minor limitation since we made a restrictive definition by only including endocarditis within three months following a SAB event. Consequently, this might have underestimated the number of endocarditis events in patients with SAB, which additionally could explain the lower frequency observed in our population compared with other studies in the field. In Paper III it would have been desirable to adjust for geographical region in the analyses since our results could have been confounded by geographical region if cases of the disease clustered by geographical region, e.g. countryside versus urban city. This would acquire detailed geo-mapping, which was outside the scope of this paper. Also it would only be relevant for non-HA SAB. Additionally, the contribution with isolates to the database could have differed by region, which we believe constitutes a minor problem since the reporting by the departments of microbiology was 90-95% during the study period.

In Paper II and Paper III, we were restricted by the rarity of SAB, which limited inclusion of covariates in the adjusted models.

Confounding by indication was not an essential problem for the main findings in Paper I and Paper III, however for some exposures of interest (e.g.: atopic dermatitis, cancer, rheumatic disease) in Paper II, it was not possible to quantify whether the increased rate of SAB was due to the pathophysiology of the disease or the treatment medication leading to the classic " chicken and the egg" causality dilemma.

Clinical implications

The interplay between host factors and acquisition of SAB is complex and far from fully understood. SAB patients often have a poor prognosis due their often multi-comorbid status prior to SAB and significant delays in the diagnosis (patient's delay and doctor's delay) increasing the likelihood of complications. The studies comprising this PhD thesis were only hypothesis-generating, but several potential risk factors for acquisition of SAB in nationwide and unselected populations were identified. An increased awareness of the disease even in young and healthy individuals is important. Although SAB is rare in young adults, our results suggest that young individuals hospitalized with SAB more frequently are of lower SES, and increased awareness should be paid to this patient category. Further, it was reassuring that once diagnosed with SAB, SES was not associated with risk of infective endocarditis. Furthermore, our results emphasizes the importance of an increased awareness of the disease, especially in children and young adults, where above one third must be considered to be at an *a priori* low risk. The findings in Paper III suggest that genetics may play a role, which should be further investigated in clinical trials and then maybe a family history of SAB will be a part of the identification of high-risk patient groups and part of the medical history of patients suspected to have bacteremia.

CONCLUSION

In this thesis we demonstrated that low socioeconomic status was inversely associated with SAB particularly in young adults (30-50 years of age), and of novelty the level of socioeconomic status was not associated with subsequent IE in patients with SAB. We further demonstrated that a significant number of risk factors, also some known from the adult population, was associated with SAB in children above 5 years and that age at time of comorbidity diagnose and time since diagnose influenced the risk of SAB for specific diseases. Importantly, above one-third of children acquiring SAB were presumably healthy prior to the infection. Finally, we showed that SAB clusters in families and having a first-degree relative previously admitted with SAB increases the risk more than six times for some family members. The increased risk was not fully explained by shared environmental factors or direct contamination among the closely related individuals.

PERSPECTIVE FOR FUTURE RESEARCH

The papers constituting this thesis were all studies of observational design, thus hypotheses-generating and therefore preclude conclusions on causality between exposures and SAB acquisition. Consequently, further studies are warranted to confirm our findings and to explore the associations in more details. It would be relevant to explore the social gradient from Paper I in more details by investigating whether specific professions are associated with increased risk of SAB and in addition whether housing influencing the risk of acquiring SAB. Also of interest would be to investigate whether working-age adults return to work full-time after the admission for SAB or whether these individuals do part time jobs or become unemployed, thus receive financial support from the government.

Further, from Paper II, it would be highly relevant to explore whether presumably healthy children were in fact healthy or the occurrence of SAB was a marker of an underlying disease. This should be further investigated by examining the cohort of presumably healthy children from the date of hospital admission with SAB and 1 year ahead to reveal the proportion of children, in which an underlying severe disease (e.g. cancer, diabetes, congenital heart disease) were diagnosed during the admission for SAB or shortly after. In children where a first episode of SAB was not a proxy for another disease, it would be interesting to conduct genome sequencing to identify potential genetic patterns, which result in increased susceptibility to *S. aureus* in a population, which is not influenced by an age-related higher comorbidity burden. In principle, this study could be extended to include all presumable healthy SAB-cases between 5 and 30 years of age, since comorbidity burden would probably be fairly low in this group.

Another essential study to be made is to reveal whether genetic risk factors observed in animal studies are also present I human. This could be investigated by identifying genetic risk factors in patients diagnosed with SAB but without established risk factors (people with high probability of genetic risk factors) and compare this group with a group of patients with a high number of risk factors but without any incident of SAB (people with low probability of genetic risk factors).

Our study about familial clustering of SAB has already led to the initiation of genetic research within the research group and in collaboration with doctors' abroad. Additionally, the statistical approach used in Paper III has primarily been used in cancer research but is a new and unique method to explore whether a well-defined infectious disease clusters in families, which can be important alone, and as a pilot study prior to large-scale, expensive genetic studies. Further, this model can be applied to other diseases. Finally, the method used in Paper III gives the opportunity to assess the hypothesis regarding familial clustering of infectious diseases and at the same time take shared environmental factors and risk of transmission into account, and we believe that the specific findings for this study will lead to studies of genetic susceptibility to SAB in the future. Future advances in the field of genetics might lead to development of new and fast diagnostic tools, new treatment modalities, and thereby improved outcome in SAB.

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APPENDICES

Appendix I (Table of codes and definitions of covariates)

Appendix I

Appendix I: CODES AND DEFINITIONS OF COVARIATES		
	Codes	Definitions
MEDICAL CONDITION		
Premature birth	Gestational week (GA) is a variable in the Medical Birth Registry	Gestational week<37 was considered premature according to guidelines
Congenital heart disease	DQ20-DQ28	
Congenital chromosome anomalies	DQ90-DQ99	
Cystic Fibrosis	DE84, 273	
Influenza	DJ09-DJ11	
Inflammatory bowel disease	DK50, DK51, DM07.4, DM07.5 569.05, 563.19, 563.01	A diagnosis of Mb. Crohn or Colitis Ulcerosa
Atopic dermatitis	DL20, 691	
Chronic heart failure	DI110, DI157, DI42, DI43, DI50	
Acute myocardial infarction	DI21, DI22 and 410	
Arrhythmias	DI44-DI49 (but not in: DI46)	
Valvular heart disease	DI351, DI371, DI361, DI342, DI352, DI360, DI370, DI372	
Periphery vascular disease	DI70, DI74, 443	
Cerebrovascular disease	DI60-DI69, 430-438	
Chronic obstructive pulmonary disease	DJ42, DJ44, 490–492	
Diabetes	DE10-DE14, 249, ACT=A10	Individuals with a diagnosis of diabetes or a prescription of glucose lowering medication
Hypertension	DI10-DI15, ACT=C02	Defined as either a diagnosis of hypertension followed by a subsequent prescription claim for an antihypertensive drug within 90 days, or as claimed prescriptions for two different classes of antihypertensive drugs.
Acute renal failure	DN17, DN19, DR34, 584	A diagnosis code of acute renal failure
Chronic renal failure	DN18, DI12, DI13, 585 (but not in: DI129, DI132)	A diagnosis code of chronic renal failure
Other renal diseases	DN03, DN04, 582, 583, 596, 588 or DI129	
Mild liver disease	DK70-DK77 (but not in: DK766, DK711, 571)	
Severe liver disease	DK766, DK711, DK704, DB150, DB160, DB190, 572, 456	
Asthma	DJ45, 493	
Chronic obstructive pulmonary disease	DJ42, DJ44, 490-492	
Psoriasis	DL40, DM070-DM073, 69609, 69610, 69619 ATC: D05AX	Psoriasis was present if an individual had a diagnosis of psoriasis or a prescription of vitamin D analogue was redeemed
Rheumatic disease	Adults: DM05, DM06, DM32-DM34, DM353, 7100, 7101, 7104, 7140, 7141, 7148, 725 Children: DI00, DI01, DI018, DI019, DM09, DM12, DM13, DM32, DM33, DL940, DL941, DM34 (but not in: DM341, DP838D, DD690B, DM797)	

Cancer	DC00-DC97, 196, 199	A diagnosis of cancer diagnosed within 5 years prior to study inclusion
HIV	DB20-DB23	
Psychiatric disease	DF2, DF3, DF4, DF50, DF60, DF93, DF982, 295, 301	Psychiatric disease where self-injuring behavior is frequently observed
Burn injury	T201, T211, T221, T231, T241, T251, T291, T202, T212, T222, T232, T242, T252, T292, T203, T213, T233, T243, T253, T293, N940-N949	A diagnosis of burn injury, thus it required contact to the emergency department or hospitalization
Bone or joint infections	DH050C, DK102C, DK102D, DM461-62, DM86, DM00, DM77 (but not in: DM869A-C)	
Soft tissue infections	DH050B, DJ383C, DE060A, DE236A, DG060A-F, DG061A + B, DG079A + B, DH000A, DH050A, DH440A, DK130A, DK140A, DK209A, DK353A + B, DK052A, DJ387G, DJ383D, DM869A-C, DH601, DJ340, DJ387, DJ391, DL983, DA064-66, DD733, DE321, DG062, DJ851, DJ390, DJ369, DK047, DK113, DK122, DM600, DL010, DL022, DL89, DS910, DS912	
INVASIVE PROCCEDURES, DEVICES AND SURGERY		
Renal replacement therapy		
Dialysis and plasmapheresis as combined variable	BJFD DZ992 BOQP0	A procedure code of dialysis or plasmapheresis and considered at risk 90 days after.
Chronic dialysis treatment	BJFD (but not in: BJFD0)	
Pacemaker	BFCA, BFCB, KFPF, KFPG (but not in: BFCA4, BFCB4, BFCA5, BFCA88, BFDB8, BFCA9)	
Prosthetic heart valves	KFMD10, KFMD11, KFMD14, KFKD10, KFGE10, KFJF10, KFJF30, KFMD20, KFMD30, KFKD20, KFGE20, KFMD00, KFKD00, KFGE00, KFJF00 KFJF20, 31269, 31129, 31130, 30729	Any type
Orthopedic device	DS52, KNJC (but not in: KNJC0), KNEJ09, KNEJ19, KNEJ29, KNEJ39, KNEJ49, KNEJ69, KNEJ79, KNEJ89, KNEJ99, KNFJ2-KNFJ9, KNFG2-KNFG9, KNFT39, KNFT49, KNFB, KNFC, 75200-75299	
Surgery overall	K (but not in: KU, KZ, KX, KY, KDHD10, KDJD30, KECB41, KEDC32, KEDC34, KEDC35, KEEC20, KEEC30, KEGC35, KNAH00, KNAH20, KNBH00, KNBH10, KNBH20, KNCH00, KNCH20, KNDH00, KNDH10, KNDH20, KNEJ09, KNFH00, KNFH20, KNGH00, KNGH10, KNGH20, KNHH00, KNHH10,	

	KNHH20, KTED00, KNAJ0, KNBJ0, KNFK0, KNGJ0, KNHJ0, KFQA, KFQB, KGDF, KJJC, KJLE, KKAS, BOQF). 322.09, 322.29, 322.50, 356.09, 472.70, 472.79, 488.40, 488.49, 574.80, 574.90	
Transplantation	KFQA, KFQB, KGDF, KJJC, KJLE, KKAS, BOQF, 32250,32229,32219, 32209	Solid organ and bone marrow
OTHER		
Parental Socioeconomic status		Highest attained educational level in parents at the date of child study inclusion.

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